CHARLES UNIVERSITY IN PRAGUE
Faculty of Science

Study programme: Clinical and Toxicological Analysis

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SYNTHESIS OF PYRIMIDO[4,5-*b*]INDOLE NUCLEOSIDES WITH MODIFIED SUGAR

Syntéza pyrimido[4,5-*b*]indolových nukleosidů s modifikovaným cukrem

Bachelor thesis

Supervisor: Prof. Michal Hocek, Ph.D., DSc.

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Declaration

The thesis was worked out at the Institute of Organic Chemistry and Biochemistry AS CR, Prague, from October 2013 to April 2014.

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In Prague, 30 May 2014

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Signature

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Abstract

Study of glycosylation reactions of base- and sugar-modified nucleosides was performed and some of the procedures were applied for the synthesis of pyrimido[4,5-*b*]indole nucleosides. Only 2′-deoxy-2′-fluoro-arabinonucleoside was successfully synthesized with nucleobase anion glycosylation. Series of 4-substituted derivatives of this nucleoside was prepared for biological activity testing.

Keywords

nucleosides, heterocycles, carbohydrates, fluorinations

Abstrakt

Byly prostudovány glykosylační reakce pyrimidoindolové báze a modifikovaných cukrů a tyto postupy byly použity pro syntézu pyrimido[4,5-*b*]indolových nukleosidů. Jediným úspěšně připraveným byl 2′-deoxy-2′-fluoro-arabinonukleosid, který byl syntetizován glykosylací pomocí aniontu báze a halogenozy. Pro testy biologické aktivity byla připravena série 4-substituovaných derivátů tohoto nukleosidu.

Klíčová slova

nukleosidy, heterocykly, cukry, fluorace

List of Abbreviations

~	Approximately
16/c	Mammary adenocarcinoma cell line
Ac	Acetyl
Bn	Benzyl
BNPP	Bis(*p*-nitrophenyl)phosphate
BSA	*N*,*O*-Bis(trimethylsilyl)acetamide
Bz	Benzoyl
cAMP	Cyclic adenosine monophosphate
CCM	Czech collection of microorganisms
CCRF-CEM	Human T cell lymphoblast-like cell line
CDK	Cyclin-dependent kinase
cGMP	Cyclic guanosine monophosphate
COSY	Correlation spectroscopy (NMR)
CPE	Cytopathic effect
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCB	2,4-Dichlorophenylmethyl
DCM	Dichloromethane
dGuo	Deoxyguanosine
DLD-1	Human colon Dukes' type C colorectal adenocarcinoma cell line
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EMEA	European medicines evaluation agency
Equiv.	Equivalents
ESI MS	Electrospray ionization mass spectrometry
Et	Ethyl
FDA	The US food and drug administration
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCT 116	Human colon colorectal carcinoma cell line
HeLaS3	Human cervical adenocarcinoma cell line
HepG2	Human liver hepatocellular cell line
HL-60	Human promyelocytic leukemia cells
HMBC	Heteronuclear multiple bond correlation (NMR)
HMDS	Hexamethyldisilazane
HR MS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence (NMR)
HSV-1	Herpes simplex virus type 1
HT-29	Human colon adenocarcinoma grade II cell line
IR	Infrared spectroscopy
J	Coupling constant (NMR)
Me	Methyl
MeCN	Acetonitrile
mp	Melting point
MRSA	Methicillin-resistant *Staphylococcus aureus*
Ms	Mesylate

NS5B	Nonstructural protein 5B
P388	Murine monocyte/macrophage cell line
RNA	Ribonucleic acid
ROESY	Rotating frame Overhauser effect spectroscopy
RP-HPFC	Reversed-phase high performance flash chromatography
RSV	Respiratory syncytial virus
RT	Room temperature
SAR	Structure – activity relationship
TDA-1	Tris[2-(2-methoxyethoxy)ethyl]amine
TMS	Tetramethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
tRNA	Transfer RNA
WiDr	Human colon colorectal adenocarcinoma cell line

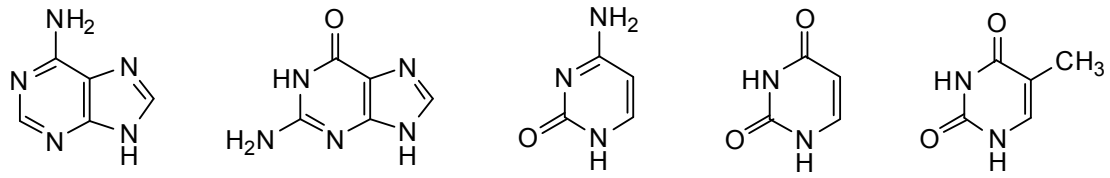
Content

1 Introduction

1.1 Biological role of nucleosides in the nature

Nucleosides and their derivatives are very important substances that participate in almost all biochemical processes in the nature. These molecules consist of nucleobase, either purine or pyrimidine and a five-carbon sugar (ribose or deoxyribose) moiety attached via β-glycosidic bond.

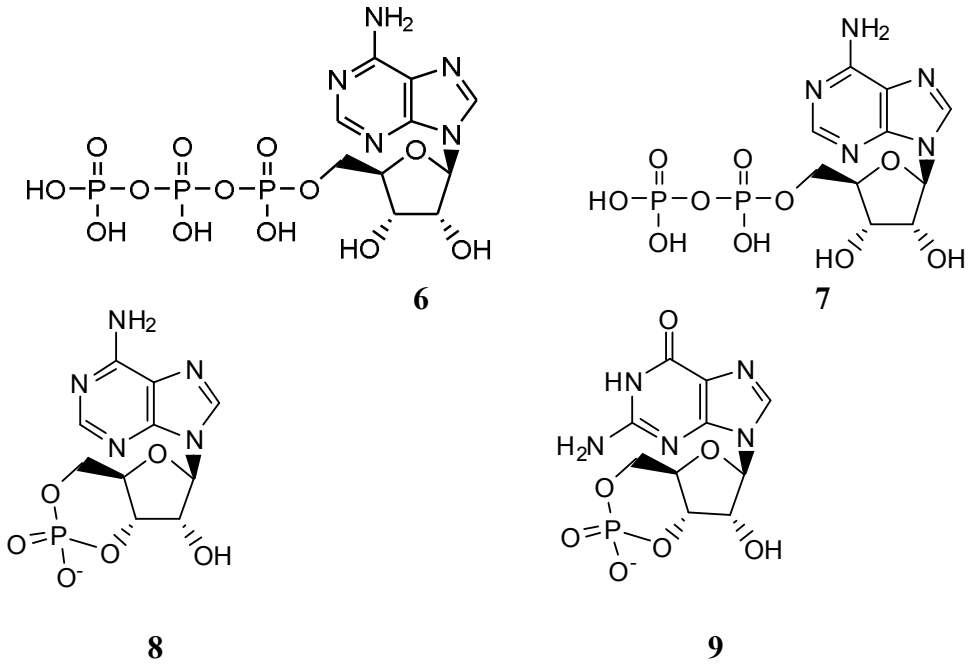
The most important nucleobases are purine derivatives – adenine (**1**) and guanine (**2**) or pyrimidine derivatives – cytosine (**3**), uracil (**4**) and thymine (**5**) (**Figure 1**). Purine and pyrimidine nucleosides form basic units of nucleic acids (DNA and RNA) which are responsible for carrying and expression of genetic information of all organisms.[1]



1 **2** **3** **4** **5**

Figure 1. Purine and pyrimidine nucleobases **1**–**5**.

Adenosine triphosphate (ATP) (**6**) is "energy-rich" macroergic compound which is able to accumulate, store, carry and release the energy required for most of the metabolic pathways. ATP is produced from adenosine diphosphate (ADP) (**7**) in metabolism processes such as photosynthesis or the biological oxidation of nutrients. Some of the nucleosides and nucleotides play an important role in the regulation of the activities of large number of metabolic processes where they function as intracellular signals. These molecules are called second messengers and some examples are cAMP (**8**) and cGMP (**9**) (**Figure 2**).[2]

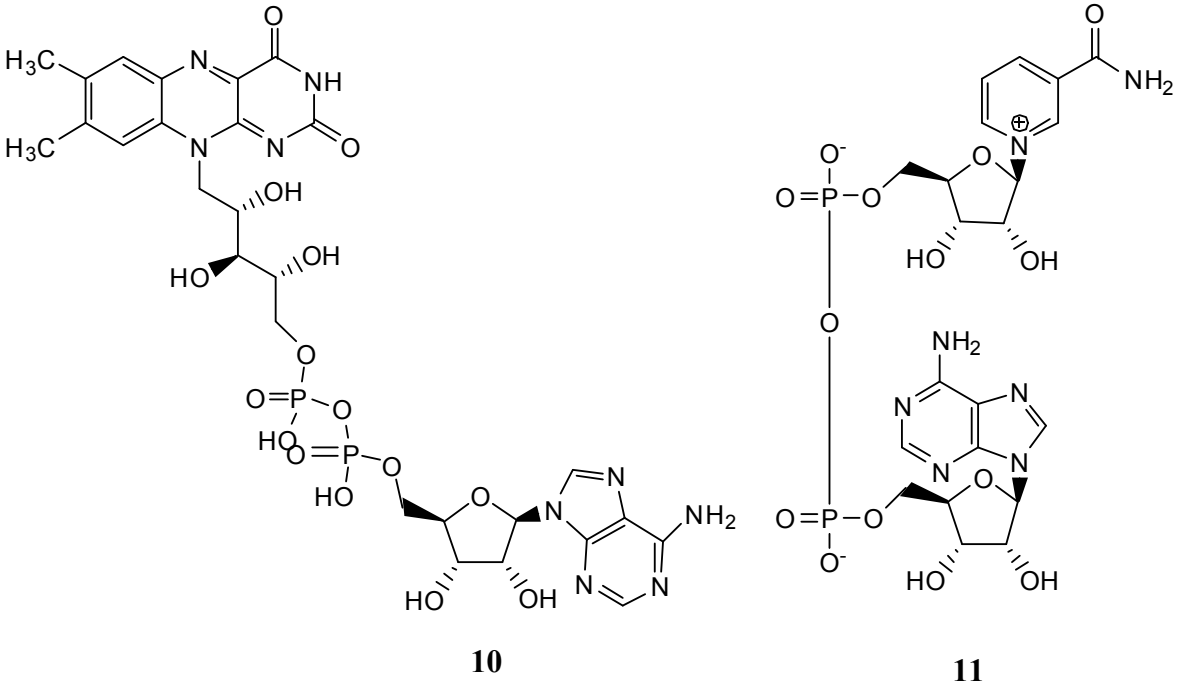


6 **7**

8 **9**

Figure 2. Macroergic compounds **6**, **7** and second messengers **8**, **9**.

Derivatives of adenosine such as flavin adenine dinucleotide (FAD^+) (**10**), nicotinamide adenine dinucleotide (NAD^+) (**11**) (**Figure 3**) and coenzyme A (**12**) (**Figure 4**) participate in many enzymatic reactions as coenzymes.[1]



10 **11**

Figure 3. NAD^+ (**10**) and FAD^+ (**11**).

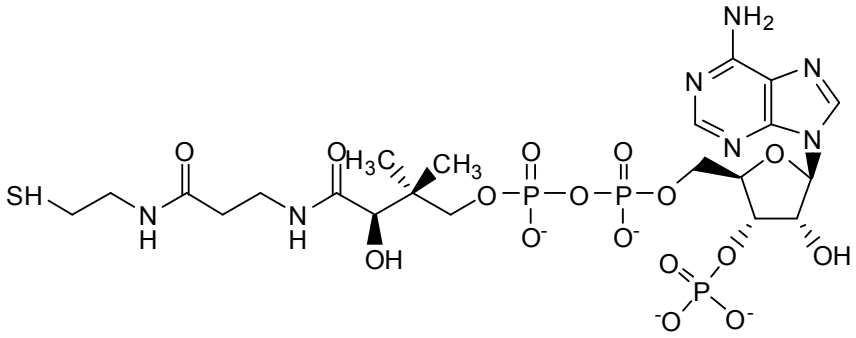


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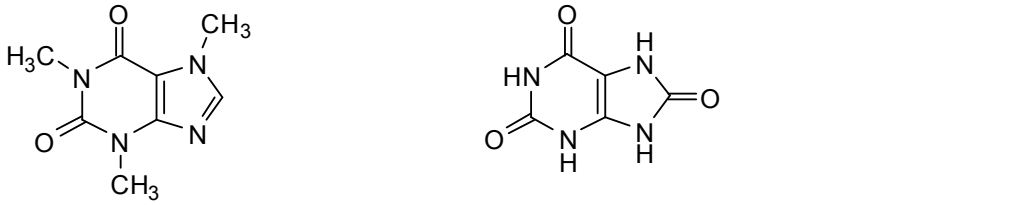
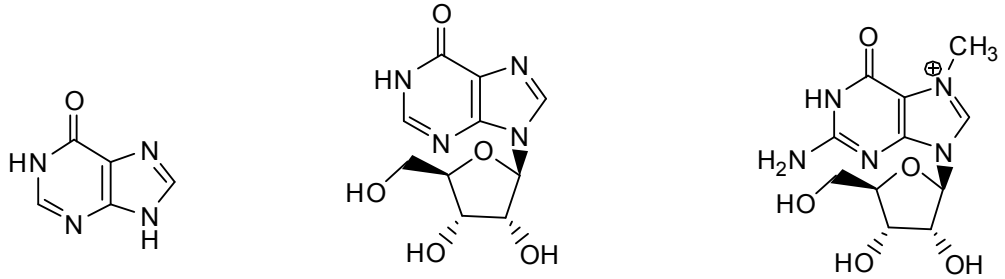
Figure 4. Coenzyme A (**12**).

Some examples of other naturally occurring purine derivatives are caffeine (**13**), uric acid (**14**), hypoxanthine (**15**) and its nucleoside inosine (**16**), which is present in tRNA and needed for codon-anticodon wooble base pairing[3] and 7-methylguanosine (**17**), modified nucleoside that occurs in variable loop of tRNA (**Figure 5**).[1]



13 **14**



15 **16** **17**

Figure 5. Naturally occuring purine derivatives **13**–**17**.

1.2 Synthetic derivatives of nucleosides

Modified nucleosides and nucleobases were one of the first chemotherapeutic agents used for the medical treatment of cancer.[4] This group of compounds includes a variety of purine and pyrimidine nucleoside derivatives which are active in both solid tumours and malignant disorders of the blood. These nucleoside analogues behave as antimetabolites, compete with natural nucleosides and induce cytotoxicity through interaction with a large number of intracellular targets. Many series of base-modified nucleosides and sugar-modified nucleosides have been synthesized and tested for biological activity against RNA viruses including HCV, Polio and Dengue virus.[5,6,7,8,9,10,11]

1.2.1 Base-modified nucleosides

Purine nucleoside analogues have been known for a long time as antimetabolites which are effectively used in the treatment of various types of cancer.[12] Mechanism of action of these compounds starts with their transport into cells and conversion to analogues of cellular nucleotides catalyzed by purine metabolic pathway enzymes.[13] First step of activation is usually monophosphorylation catalyzed by nucleoside kinases.[14,15] Phosphorylated metabolites then interfere with enzymes of DNA replication and thus block DNA synthesis which results in damage of DNA chain and causes apoptosis of the cell. Some of FDA approved purine antimetabolites are cladribine (**18**), fludarabine which is administered as soluble monophosphate – fludara (**19**) and nelarabine (**20**) (**Figure 6**).[12]

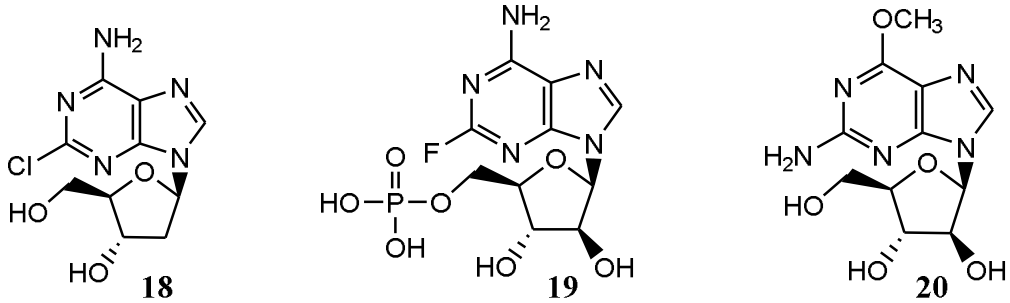
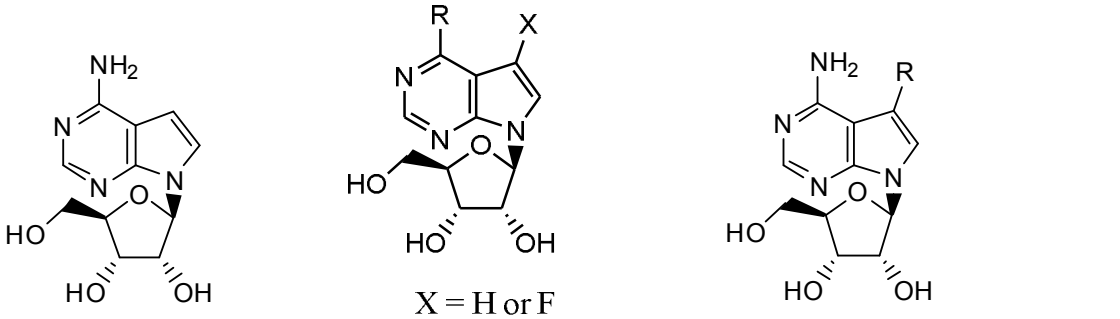


Figure 6. Purine antimetabolites **18**–**20**.

Cladribine (**18**) is used mainly for treatment of hairy-cell leukemia,[4] non-Hodgkin lymphoma[4] and chronic lymphocytic leukemia.[16] Fludarabine (**19**) treats chronic lymphocytic leukemia[16] and nelarabine (**20**) is used for the treatment of T cell malignancies.[17]

Other two interesting classes of nucleosides with potent cytotoxic effect against cancer cell lines were developed in our group. 6-hetaryl-7-deazapurine ribonucleosides **22**[11] and 7-hetaryl-7-deazaadenosines **23**[18] are derivatives of tubericidin (**21**). The highest potential in these studies showed 7-H or 7-F derivatives of 6-furyl- or 6-thienyl-7-deazapurines **22** exerting nanomolar *in vitro* cytostatic activities against a wide panel of leukemia or cancer cell lines (**Figure 7**).[11]

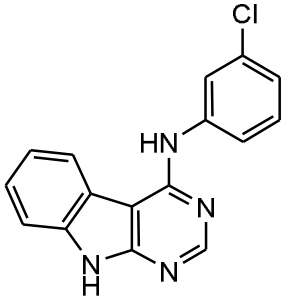


21 **22** **23**

Figure 7. Tubericidin (**21**) and derived ribonucleosides **22**, **23**.

Derivatives of 7-hetaryl-7-deazaadenosine series **23** bearing 5-membered heterocycles at position 7 showed promising *in vitro* antiproliferative effects towards broad range of hematological or solid tumor cell lines and cytostatic effects comparable to clofarabine (**45**) (**Figure 13**, **page 17**). Both of these classes of nucleosides also showed high but non-specific anti-HCV activities.[11,18]

An interesting group of nucleobases are pyrimidoindoles **24**–**26**, which are benzo-fused analogues of 7-deazapurines and some of these compounds were reported as tyrosine kinase inhibitors with nanomolar activity (**Figure 8**) .[19,20]



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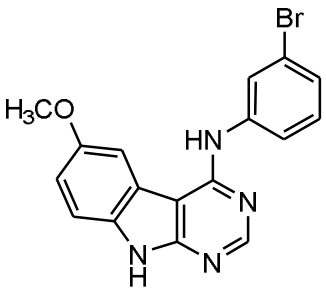

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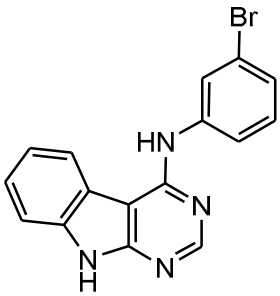

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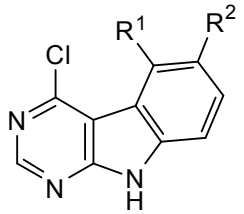
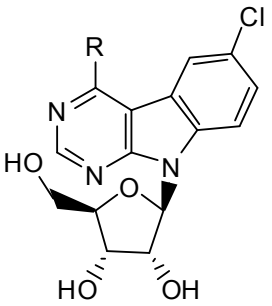
Figure 8. Some examples of pyrimidoindoles **24**–**26**.

Synthesis of three halogenated pyrimidoindole bases **27**–**29** and corresponding ribonucleosides was reported.[7,8] Compounds **30**–**32** exerted interesting anti-Dengue virus or anti-HCV activities, but in some cases accompanied by cytotoxicity (**Figure 9**). Compared to 6-hetaryl-7-deazapurine **22** and 7-hetaryl-7-deazaadenosine ribonucleosides **23** (**Figure 7**) more specific anti-HCV activity but no significant activity against leukemia or cancer cell lines was observed for pyrimidoindole ribonucleosides **30**–**32**.

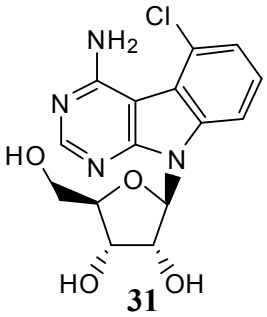


27 R^1 = H; R^2 = H
28 R^1 = Cl; R^2 = H
29 R^1 = H; R^2 = Cl



R = 2-furyl, 2-thienyl or 2-benzofuryl

30



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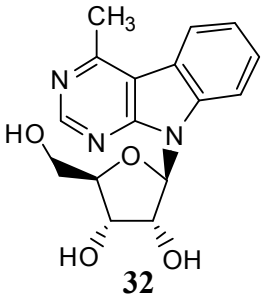

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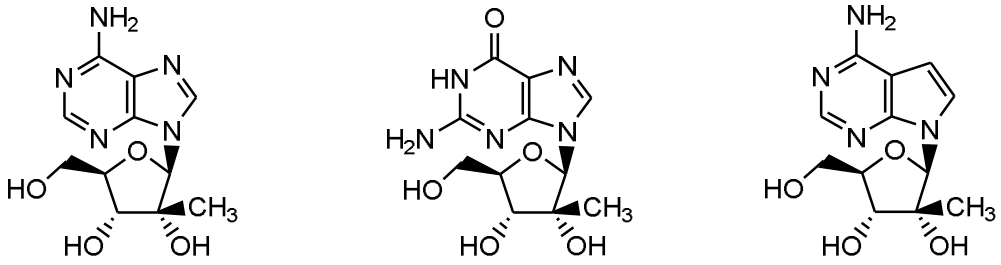
Figure 9. Pyrimidoindole bases **27**–**29** and biologically active ribonucleosides **30**–**32**.

1.2.2 Sugar-modified nucleosides

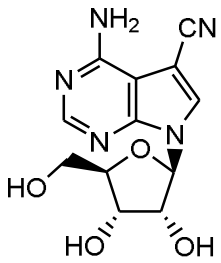
HCV infection is the most common infection causing liver disease worldwide with more than 180 million people infected.[21] This infection is caused by blood-borne RNA virus from the flavivirus family[22] and it can cause both acute and chronic hepatitis infection. Chronic infection can lead to cirrhosis of the liver and HCC.[23] Several modified purine nucleosides, such as 2′-β-*C*-methyl ribonucleosides were found to show significant anti-HCV activity.[24] Eldrup *et al.* reported synthesis and SAR of purine ribonucleosides as inhibitors of HCV RNA replication.[25] 2′-β-*C*-Methyladenosine (**33**) and 2′-β-*C*-methylguanosine (**34**) (**Figure 10**) were reported as potent and selective inhibitors of HCV RNA replication, and their triphosphate forms were found to be active in the inhibition of HCV NS5B-mediated RNA synthesis. Base modified analogue of (**33**) 2′-β-*C*-methyl-7-deazaadenosine (**35**) showed promising antiviral activity and *in vivo* pharmacokinetics were successfully tested in three animal species.[26]



33 **34** **35**

Figure 10. 2′-β-*C*-Methyl ribonucleosides **33**–**35** with anti-HCV activity.

Other examples include 2′-β-*C*-methyl nucleoside analogues of toyocamycin (**36**) and sangivamycin (**37**), nucleoside antibiotics occuring in the nature. These derivatives (**Figure 11**) were synthesized in an effort to increase biological activity of their original compounds[27] and some of the derivatives **38**–**43** showed excellent anti-HCV activities (0.5–100 μM for EC_{50}).[28]



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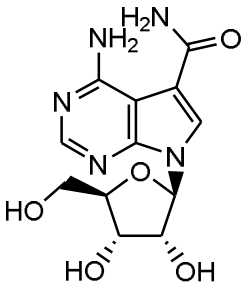

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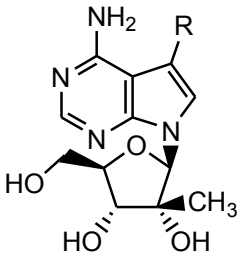
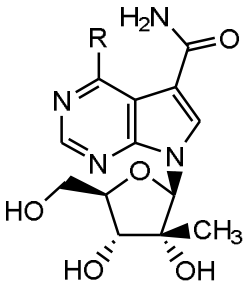


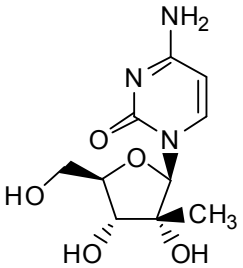
38 R = $CONH_2$
39 R = $CNHOCH_3$
40 R = CNHNHOH



41 R = $NHCH_3$
42 R = $N(CH_3)_2$
43 R = $NHOCH_3$

Figure 11. Toyocamycin (**36**), sangivamycin (**37**) and derived nucleosides **38**–**43**.

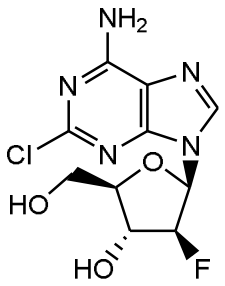
Noroviruses are today recognized as the leading cause of epidemics of gastroenteritis and cause of sporadic gastroenteritis affecting millions of people. This illness is common cause of hospitalization for gastroenteritis, however, it can be sometimes fatal, especially among young children and the elderly.[29] A nucleoside polymerase inhibitor of HCV, 2′-β-*C*-methylcytidine (**44**) (**Figure 12**), was reported as an inhibitor of the *in vitro* replication of (murine) norovirus.[30] It has been shown that it inhibits virus-induced CPE formation, viral RNA synthesis and infectious progeny formation with EC_{50} values of ~2 μM.



44

Figure 12. 2′-β-*C*-Methylcytidine (**44**).

Other class of sugar-modified nucleosides with significant cytostatic activities are deoxyadenosine analogues bearing fluorine atom in the 2′-arabino moiety. 2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabino-furanosyl) adenine (**45**) (**Figure 13**) commercially known as clofarabine was synthesized and its activity was tested against several leukemic cell lines including those of human origin.[31] To exert its cytotoxic effect, clofarabine requires intracellular phosphorylation by DCK[32] and its triphosphate form **46** is an inhibitor of both DNA polymerase α (pol α) and ribonucleotide reductase.[33,34] Clofarabine (**45**) showed potent antiproliferative activity against four human colon tumor cell lines (HCT116, HT-29, DLD-1, WiDr), with IC_{50} values of 0.26 μM during 72 hrs exposure.[31] Furthermore, it exhibited *in vivo* antitumor activity against following murine tumors: P388 leukemia, colon 36 and mammary 16/c.[35] It also cured both early- and advanced-stage colon 36. In other study, 25% of pediatric patients with relapsed or refractory leukemia achieved complete remission by intravenous treatment with clofarabine.[36]



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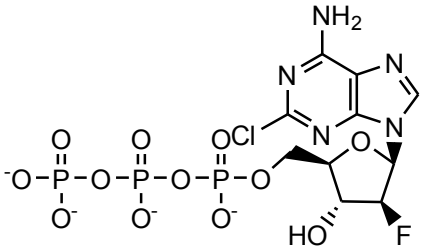

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Figure 13. Clofarabine (**45**) and its triphosphate form **46**.

Inserting two fluorine atoms into the deoxyribose ring of deoxycytidine led to synthesis of gemcitabine (**47**) (2′,2′-difluorodeoxycytidine) (**Figure 14**).[37] This prodrug is inactive in its original form and gains biological activity by intracellular phosphorylation.[38] There are few reasons for the significant activity of gemcitabine (**47**): it is actively transported into cells through cell membrane, its phosphorylation is more efficient and elimination at a slower rate. Gemcitabine's cytotoxic activity against cancer cells is achieved by incorporation into growing DNA chain and disruption of further DNA synthesis.[39] This causes the apoptosis of the cell. The potent cytotoxic activity of gemcitabine was revealed during studies on murine and human cell lines,

solid murine tumors and human tumor xenografts.[40,41] Later on, treatment of patients with wide variety of tumors started with approval both by the FDA and the EMEA. This includes non-small lung cancer,[42,43] breast cancer,[44] adenocarcinoma of the pankreas,[45,46,47,48] bladder cancer and ovarian cancer.[38]

47 **48**

Figure 14. Gemcitabine (**47**) and 2′,2′-difluorodeoxyguanosine (**48**).

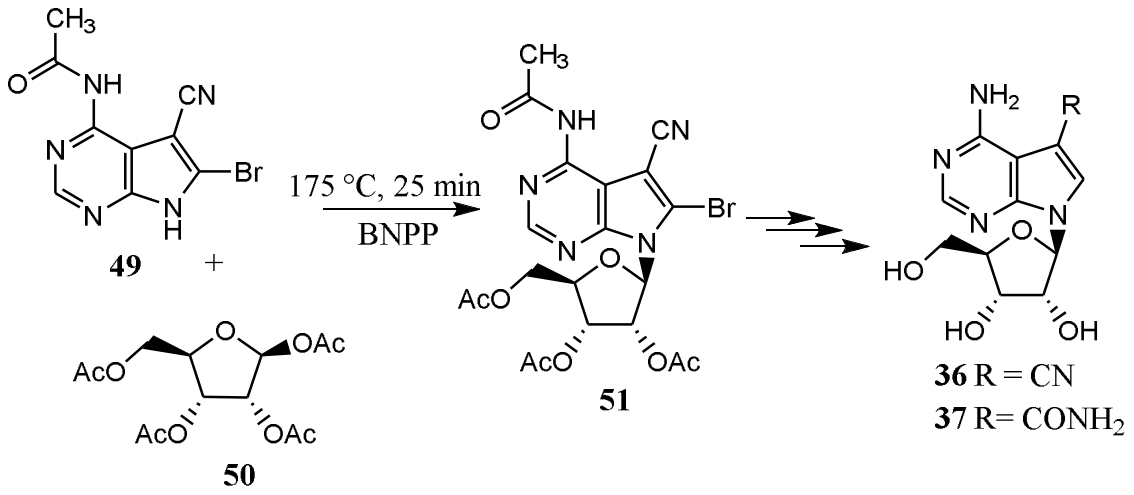
Inspired by clinical success of gemcitabine (**47**), cytotoxicity, metabolism, and mechanisms of action of 2′,2′-difluorodeoxyguanosine (**48**) (**Figure 14**) were studied in Chinese hamster ovary cells.[49] This nucleoside **48** was found to be activated by dGuo kinase and showed inhibitory effects on ribonucleotide reductase and DNA synthesis.

1.3 Synthetic approach towards nucleosides

Extraordinary biological activity of naturally occurring 7-deazapurine nucleosides and their derivatives caused interest in design and synthesis of novel types of nucleosides. Many studies on synthesis of 7-deazapurine nucleosides have been done in recent years in order to upgrade their properties. Different types of reactions for creating glycosidic bond between nucleobase and sugar derivatives have been introduced and optimized. Among them, the most commonly used nucleoside forming reactions are Silyl-Hilbert-Johnson reaction and the nucleobase anion glycosylation. Other mentionable examples of glycosylations are the fusion reaction and the metal salt procedure, both suffering from low yields.

1.3.1 The fusion reaction

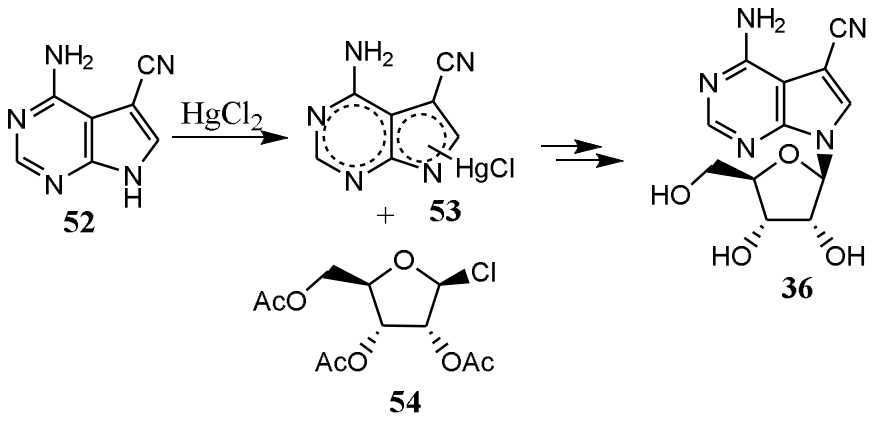
The fusion reaction between nucleobase and peracylated ribose is usually performed at temperature around 160 °C with Lewis acid as catalyst. This reaction was used for the first total synthesis of toyocamycin (**36**) and sangivamycin (**37**).[50] Acetylated nucleoside **51** was prepared by heating the mixture of 4-acetamido-6-bromo-5-cyanopyrrolo-[2,3-*d*]pyrimidine (**49**) and protected ribofuranose **50** at 175 °C in the presence of bis(*p*-nitrophenyl)phosphate (**Scheme 1**). This intermediate **51** was subsequently converted to target compounds **36**, **37** with series of reactions. This procedure is not usually used for the synthesis of nucleosides since it requires high temperature and affords only low yields of desired product.



Scheme 1. The fusion reaction.

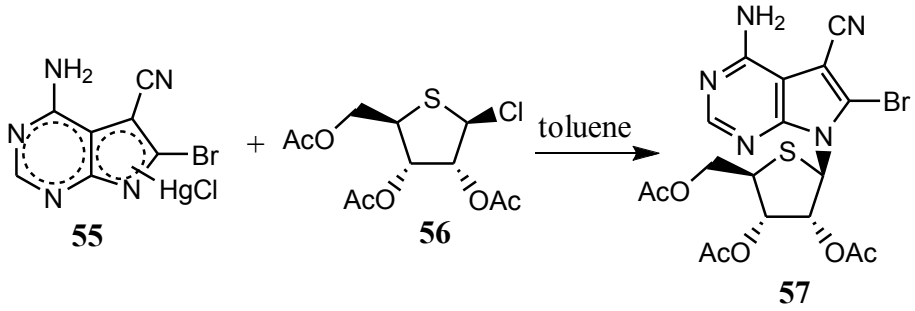
1.3.2 The metal salt procedure

In this type of glycosylation the metal salt of nucleobase reacts with protected sugar halide. Treatment of 4-amino-5-cyanopyrrolo[2,3-*d*]-pyrimidine (**52**) with mercuric chloride in aqeous sodium hydroxide solution afforded chlormercury derivative **53** which reacted with halogenose **54** and deacetylation furnished target nucleoside **36** in low yield (**Scheme 2**).[51]



Scheme 2. Toyocamycin (**36**) prepared by metal salt procedure.

This procedure was also used for synthesis of 4'-thio analogues of toyocamycin (**36**).[52] Chloromercury salt **55** of pyrollopyrimidine **66** reacted with halogenose **56** in dry toluene to furnish the protected intermediate **57** in 25 % yield (**Scheme 3**). Toxicity of used mercury compounds accompanied by low yields, make this method rare in the nucleoside synthesis.



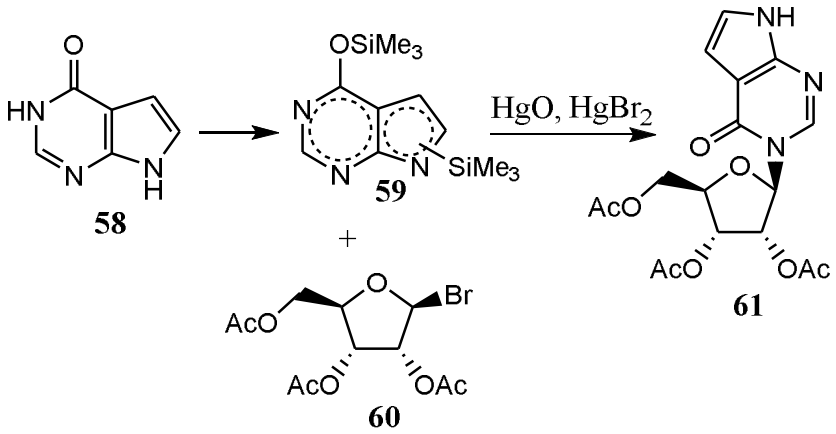
Scheme 3. Synthesis of 4'-thio analogue **57** of toyocamycin (**36**).

1.3.3 Silyl-Hilbert-Johnson reaction

Silyl-Hilbert-Johnson reaction has been known as the most common method for glycosylation of nucleobases. Nucleoside is formed in the reaction of silylated nucleobase with electrophilic sugar derivative in the presence of catalyst. Several different protocols are used for this procedure.

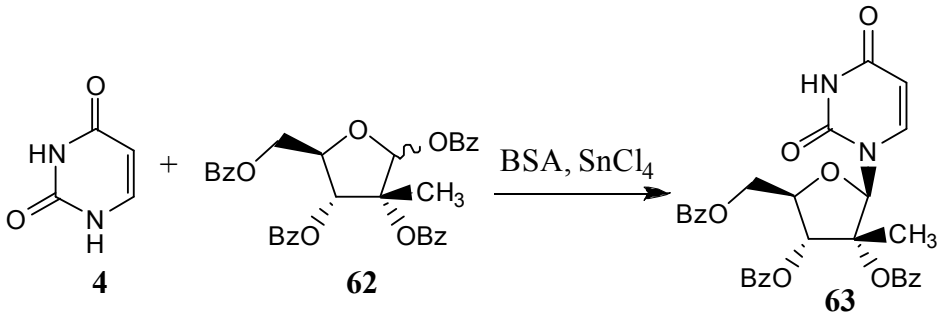
Wittenberg procedure is performed in the presence of mercuric oxide and was successfully used for synthesis of protected pyrrolopyrimidine ribonucleosides.[53] This two-step procedure starts with silylation of nucleobase **58** by HMDS in the presence of $(NH_4)_2SO_4$ and is followed by second step – reaction of **59** with bromose **60** catalyzed

by mixture of mercuric oxide and mercuric bromide to furnish N-1 nucleoside **61** exclusively (**Scheme 4**).



Scheme 4. Wittenberg procedure for N-1 nucleoside **61**.

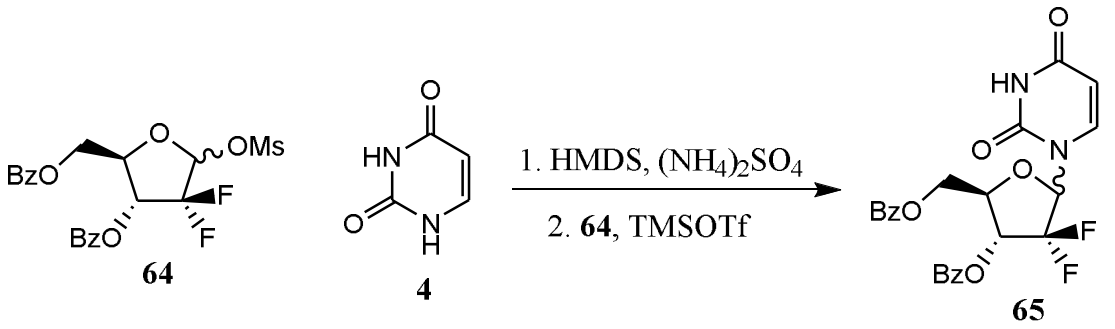
In the synthesis of 2′-β-*C*-methyluridine,[54] $SnCl_4$ was used as catalyst. One-pot reaction was performed using BSA (*N*,*O*-Bis(trimethylsilyl)acetamide) as silylation agent and reaction of uracil (**4**) with ribose **62** afforded desired protected nucleoside **63** in 57 % yield (**Scheme 5**).



Scheme 5. Synthesis of 2′-β-*C*-methyluridine (**63**).

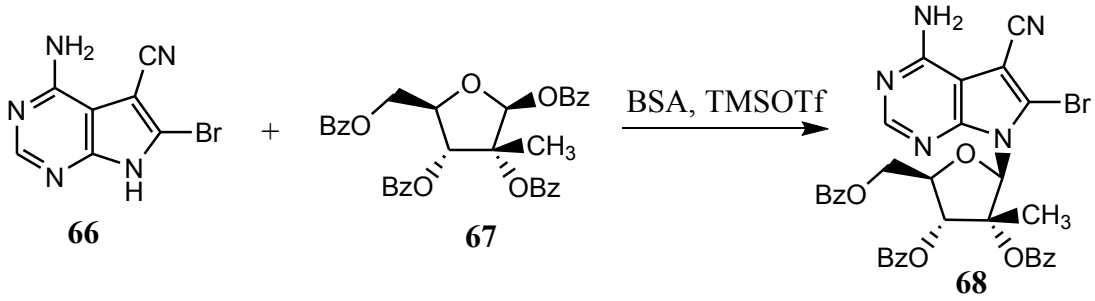
Other successfully used Lewis acid catalyst is TMSOTf. Using this conditions, acyloxonium ion is formed as intermediate. Nucleophilic attack of nucleobase is forced from the opposite, β-face of the molecule according to Baker's trans rule which leads to β-D-nucleoside exclusively. Various 2′-deoxy-2′,2′-difluoro-L-erythro pentofuranosyl-purine and pyrimidine nucleosides were synthesized by two-step Silyl-Herbert-Johnson reaction.[55] In this study silylation of uracil **(4)** by excess of HMDS in the presence of $(NH_4)_2SO_4$ was followed by addition of previously mesylated compound **64** and slow

addition of TMSOTf. After 10 h reflux at 90–100 °C under argon, anomeric mixture of target nucleoside **65** was obtained (**Scheme 6**).



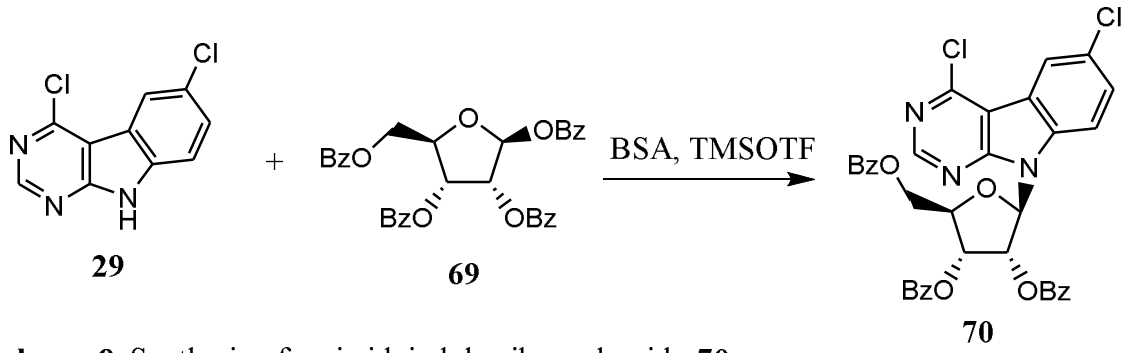
Scheme 6. Synthesis of 2′-deoxy-2′,2′-difluoro-L-erythro-pentofuranosyl nucleoside **65**.

In the synthesis of 2′-β-*C*-methyl toyocamycin and sangivamycin analogues (**Figure 11**), nucleobase **66** was first silylated with BSA at RT and then reacted with 1 equiv. of protected sugar **67** in the presence of 3 equiv. of TMSOTf (**Scheme 7**).[28] Nucleoside **68** was obtained in 75 % yield. This improved procedure known as Vorbrüggen reaction is the most common method used for the synthesis of nucleosides.



Scheme 7. Vorbrüggen reaction using BSA and TMSOTf.

Analogous protocol was used in the synthesis of pyrimidoindole ribonucleosides.[7] After 10 min silylation of **29** with BSA at RT, TMSOTf and ribofuranose **69** were added (**Scheme 8**). After 8 h heating at 60 °C, desired nucleoside **70** was obtained in 54 % yield.

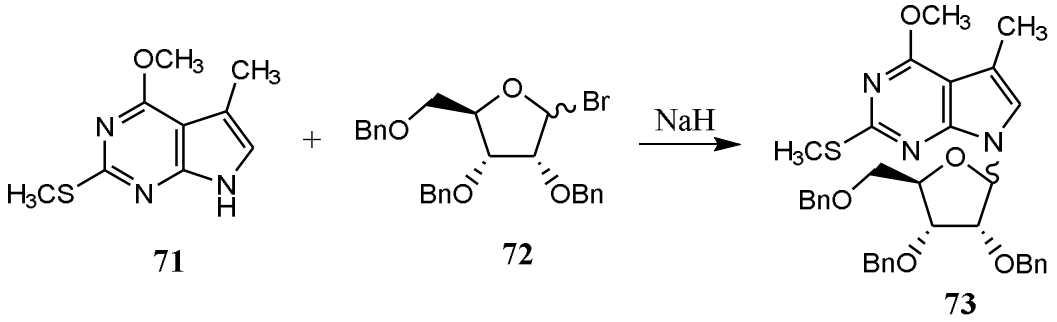


Scheme 8. Synthesis of pyrimidoindole ribonucleoside **70**.

1.3.4 Nucleobase anion glycosylation

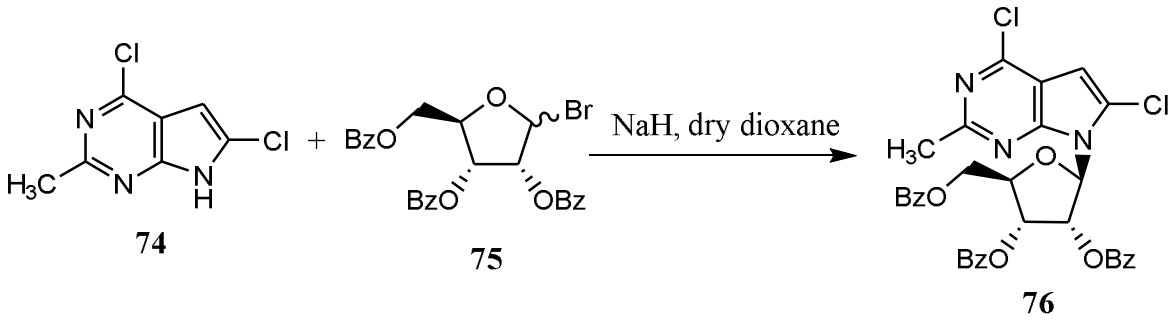
This procedure requires the presence of a base (NaH or KOH) which forms anion of nucleobase by its deprotonation. Glycosidic bond is formed by reaction of this anion with electrophilic sugar derivative (for example halide or mesylate).

Sodium salt procedure was used to generate anion in the presence of NaH.[56] Following glycosylation of nucleobase anion of **71** with benzyl-protected ribofuranose **72** gave the mixture of α- (25 %) and β-D-anomers (25 %) of target nucleoside **73** (**Figure 9**).



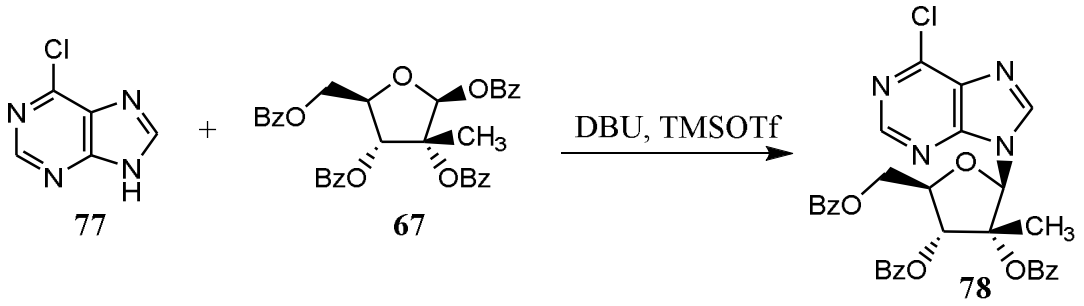
Scheme 9. Sodium salt procedure using NaH.

Glycosylation of benzoyl-protected bromose **74** and the nucleobase anion of **75** formed by NaH in dry dioxane led to nucleoside **76** in excellent 91 % yield (**Scheme 10**).[53]



Scheme 10. Sodium salt procedure using NaH and dry dioxane as solvent.

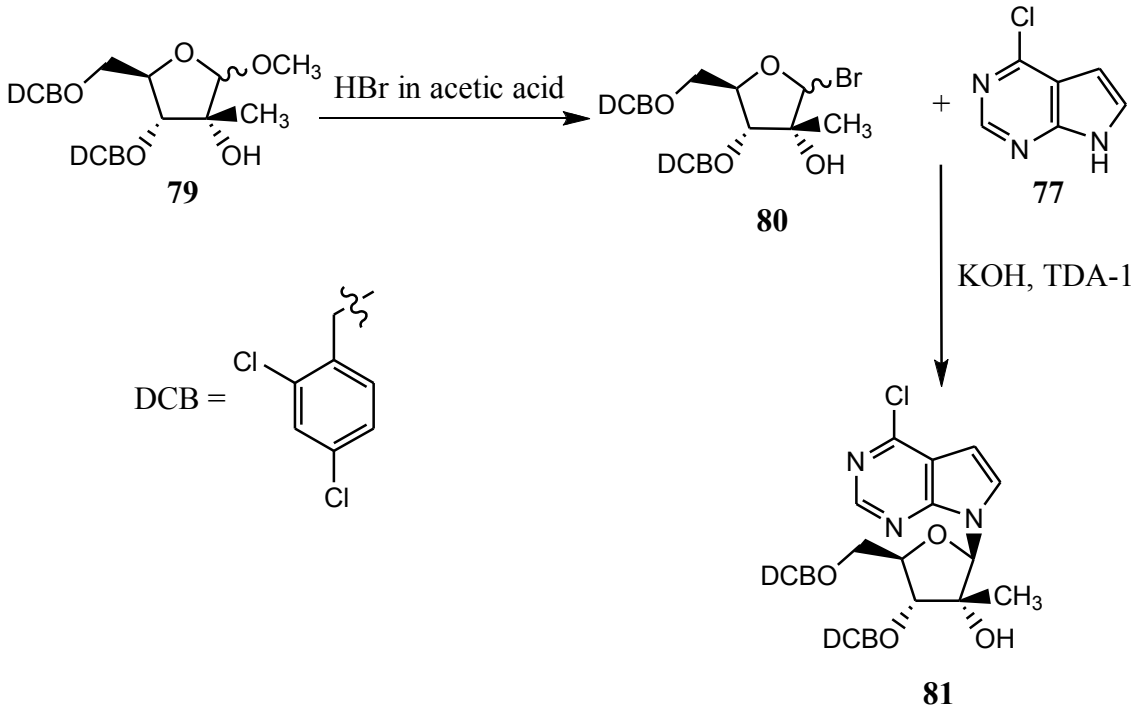
In other Vorbrüggen-type condensation, DBU is used as base. In this modified procedure TMSOTf is added slowly to a precooled mixture of 6-chloropurine (**77**), ribose **67** and DBU. Reaction mixture is heated to 60 °C for 4 h giving the protected nucleoside **78** in 83 % yield (**Scheme 11**).[57]



Scheme 11. Glycosylation of 6-chloropurine (**77**) using DBU and TMSOTf.

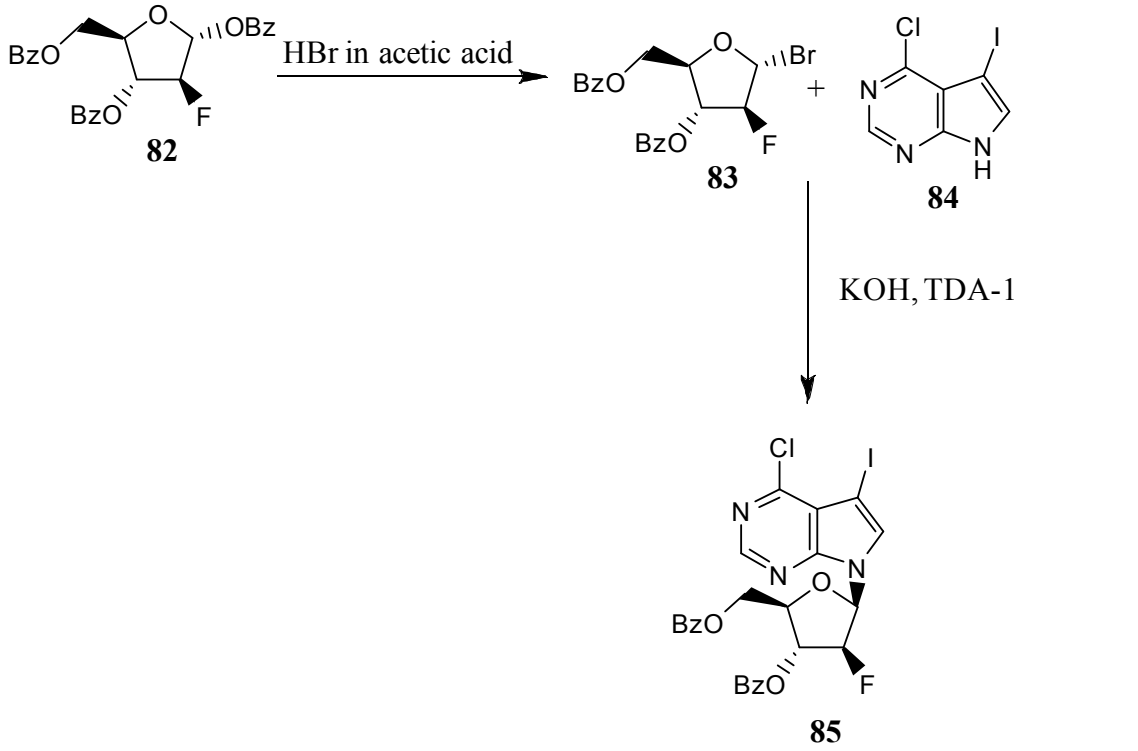
For nucleobase anion glycosylation under solid-liquid conditions powdered KOH and TDA-1 as catalyst are required. Reaction is usually performed in MeCN or toluene. When sugar halide in α-configuration is used, this reaction proceeds stereoselectively to give β-D-nucleosides.[53]

This procedure was used in the synthesis of potential HCV RNA replication inhibitors.[9] 2′-β-*C*-Methyl ribofuranose **79** was synthesized and converted to the corresponding 1-bromo sugar halide **80** which then reacted with sodium salt of **71** to give the β-anomer **81** (**Scheme 12**).



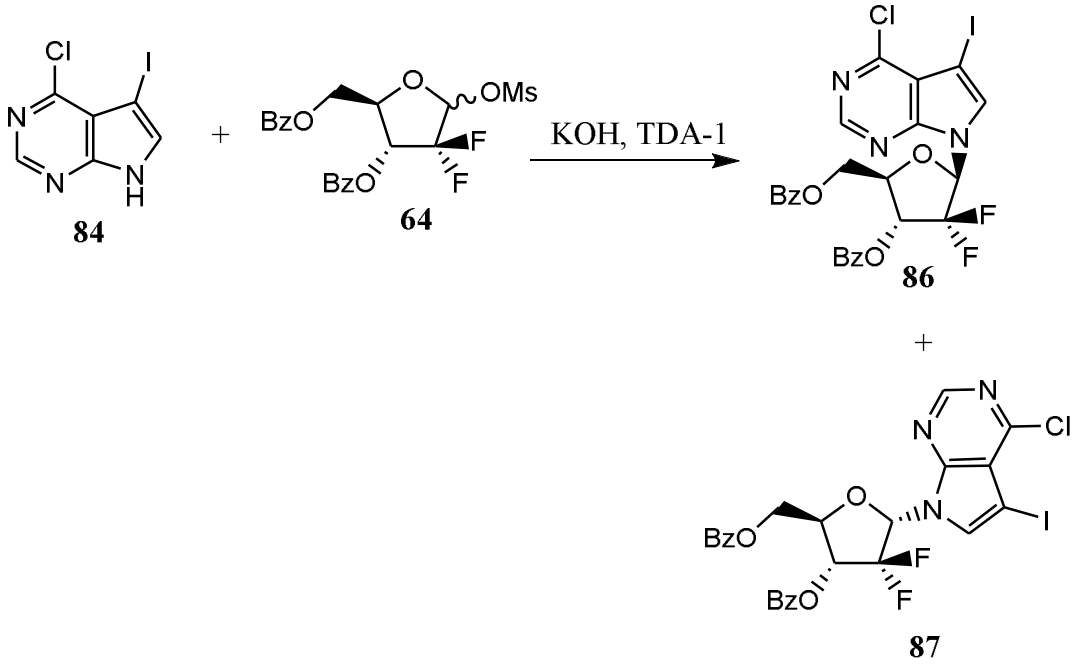
Scheme 12. Synthesis of 2′-β-*C*-methyl nucleoside **81**.

Conversion of **82** to bromide **83** and anion glycosylation was also used in the synthesis of 7-deazaadenine 2′-deoxy-2′-fluoroarabino nucleosides **85** (**Scheme 13**).[58]



Scheme 13. Synthesis of 7-deazaadenine 2'-deoxy-2'-fluoroarabino nucleoside **85**.

Further, nucleobase anion glycosylation procedure was used for the synthesis of 7-deazapurine 2′-deoxy-2′,2′-difluoro ribonucleosides **86** from difluorosugar mesylate **64**.[59,60] Reaction was performed on a large scale (10 g of nucleobase **84**) with 1 equiv. of TDA-1 and 2 equiv. of KOH (**Scheme 14**). Reaction mixture was heated at 50 °C for 4 days and afforded mixture of anomers **86** and **87** (α/β 1:2) in 12 % yield.



Scheme 14. Synthesis of 7-deazapurine 2′-deoxy-2′,2′-difluoro ribonucleoside **86**.

2 Aims of the thesis

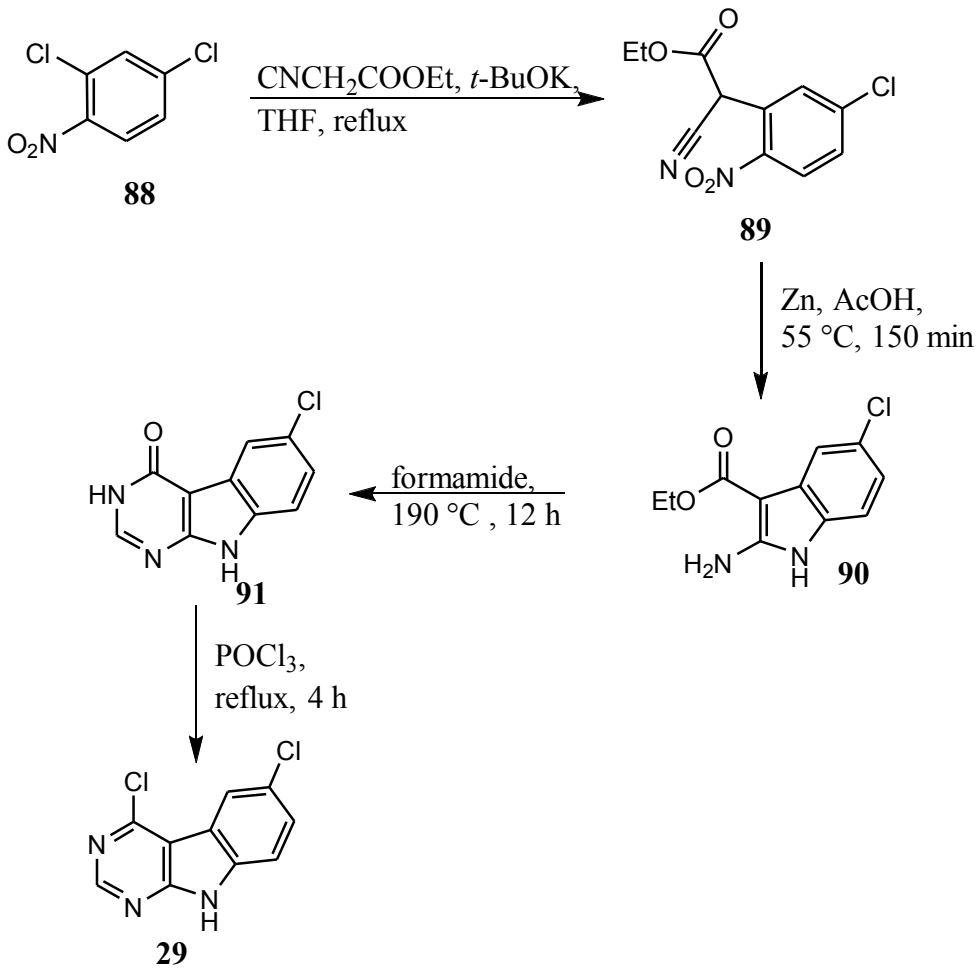
1. Study of glycosylation reactions of pyrimido[4,5-*b*]indoles with diverse modified ribose derivatives such as 2-*C*-methyl-β-D-ribofuranose, 2-deoxy-2-fluoro-α-D-arabinose and 2-deoxy-2,2-difluoro-D-ribofuranose.
2. In successful cases, synthesis of several base-modified nucleoside derivatives for biological activity testing.

3 Results and discussion

Interest in the synthesis of pyrimido[4,5-*b*]indole nucleosides was based on the knowledge that some of the corresponding ribonucleosides **30**–**32** showed promissing anti-HCV and anti-Dengue activity,[7,8] 2′-*C*-methylribonucleosides analogues **33**–**35** of adenosine and 7-deazapurine are active against HCV.[25,28] Clofarabine (**45**) with fluorine atom at 2′ position in the deoxyribose moiety is used in the cancer treatment[12,36] and gemcitabine (**47**) (2′-2′-difluorodeoxycytidine) treats various types of malignant tumors.[38] Therefore, to complement the SAR for this class of compounds, 2′-*C*-methylribo, 2′-deoxy-2′-fluoro and 2′-deoxy-2′,2′-difluororibo sugar-modified analogues of pyrimido[4,5-*b*]indole ribonucleosides were the aim of this work.

3.1 Nucleobase synthesis

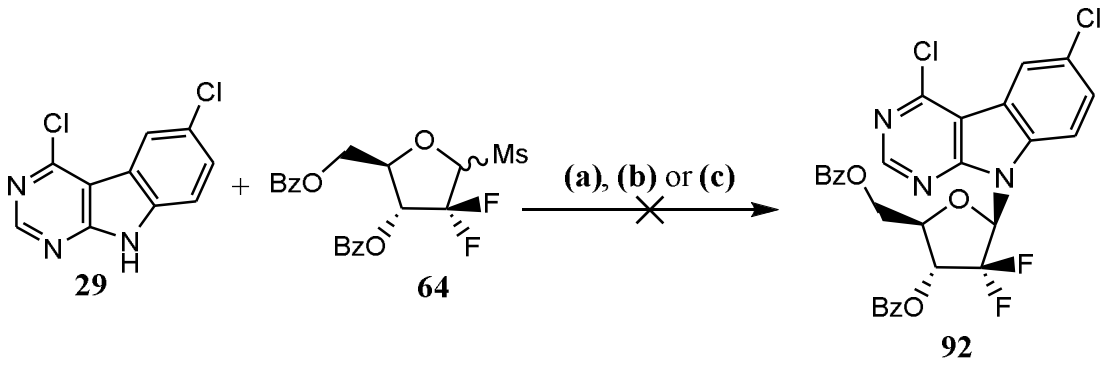
Synthesis of target nucleosides started with the construction of 4,6-di-chloro-9*H*-pyrimido[4,5-*b*]indole (**29**) following published procedure for synthesis of this heterocyclic nucleobase.[7] First step was the synthesis of ethyl-2-(2-nitrophenyl)-cyanoacetate (**89**) from 2,4-dichloronitrobenzene (**88**) and cyanoacetate according to the reported procedure.[61] Reduction of **89** with zinc dust in acetic acid followed by spontaneous cyclization afforded indole derivative **90** which gave keto-base **91** after cyclocondensation with formamide. In the final step, compound **91** was converted to dichloropyrimidoindole **29** by treatment with $POCl_3$ under reflex (**Scheme 15**). Nucleobase **29** was synthesized by this 4-step procedure in 31 % overall yield with no chromatographic purification needed.



Scheme 15. Synthesis of 4,6-dichloropyrimidoindole **29**.

3.2 Attempts at the synthesis of 2'-deoxy-2',2'-difluororibonucleoside

The first sugar derivative tried for glycosylation with 4,6-dichloro-9*H*-pyrimido-[4,5-*b*]indole (**29**) was benzoyl-protected 2-deoxy-2,2-difluoro-D-erythro-pentofuranosyl-1-mesylate (**64**) (**Scheme 16**).



Scheme 16. Glycosylation of base **29** with mesylate **64**. Reagents: **(a)** KOH (2 equiv.), TDA-1 (1 equiv.), MeCN; **(b)** BSA (1 equiv.), TMSOTf (2 equiv.), MeCN; **(c)** NaH (2 equiv.), MeCN.

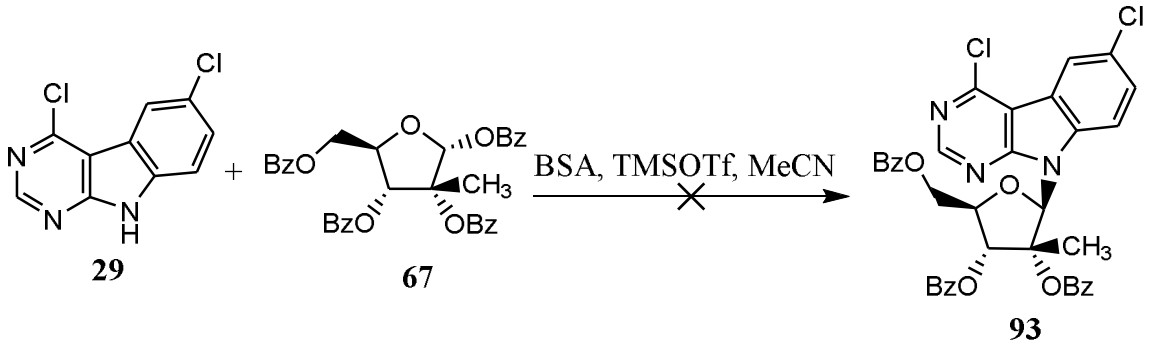
Three different types of reactions were applied. Nucleobase anion glycosylation was previously used for the synthesis of 2'-deoxy-2',2'-difluororibonucleosides,[59,60] thus this published procedure using KOH and TDA-1 at 50 °C was followed. Under these conditions no product was observed, only few decomposed sugar fractions and nucleobase **29** with starting sugar material **64** was recovered. Highering temperature to 80 °C gave the same result with no product observed. Therefore, Vorbrüggen conditions were tried out. Silylation of **29** by BSA for 1 h at 80 °C followed by addition of TMSOTf and mesylate **64** and stirring at 80 °C for 1 day and at RT for 3 more days did not lead to desired nucleoside **92**. Again, decomposed sugar fractions were only observed products and unreacted nucleobase **29** and some mesylate **64** were recovered. Sodium salt procedure using NaH was last tried option. Mixture of nucleobase **29** and NaH in MeCN was stirred at 80 °C for 1 h. and mesylate **64** was added by parts. After 1 day stirring at 80 °C no product was observed, only decomposed fractions of sugar and unreacted sugar **64** and nucleobase **29** were obtained. All these experiments are summarized in **Table 1**.

Table 1. Results of glycosylation reactions of **29** with **64**.

Entry	Reagents	Equiv. of **64**	Temp.	Time	Yield of **92**
1	**(a)**	3	50 °C	4 days	–
2	**(a)**	3	80 °C	4 days	–
3	**(b)**	3	80 °C	1 day (3 more at RT)	–
4	**(c)**	3	80 °C	1 day (3 more at RT)	–

3.3 Attempts at the synthesis of 2'-C-methylribonucleoside

The second sugar derivative used for glycosylation with **29** was 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranose (**67**) (**Scheme 17**).



Scheme 17. Vorbrüggen reaction of pyrimidoindole **29** with 2-C-methyl ribose **67** derivative.

One pot silylation using Vorbrügen conditions was sucessfully applied in the synthesis of pyrimido[4,5-*b*]indole ribonucleosides[7, 8] and therefore, this procedure was chosen for the synthesis of **93**. Nucleobase **29** was silylated by BSA for 30 min at RT and TMSOTf with ribofuranose **67** was added. Reaction mixture was heated to 70 °C and after 3 h starting sugar material **67** dissapeared but no target product **93** was observed. Unreacted nucleobase **29** and few fractions of decomposed sugar were obtained. Higher temperature (85 °C) and stirring for 1 day gave the same results. Silylation at 60 °C for 30 min was tried out as attempt to increase amount of silylated nucleobase. Addition of **67** and TMSOTf (dropwise) and stirring at 80 °C for 18 h led again to decomposed sugar fractions and recovered nucleobase **29**. Unsuccessful

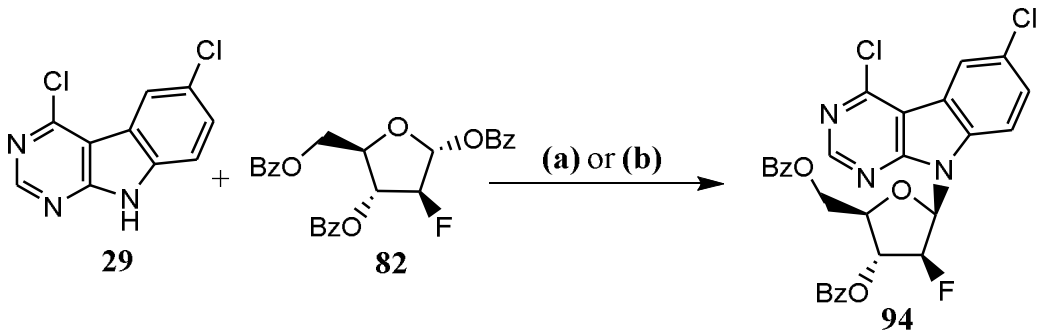
attempts with tried conditions are summarized in **Table 2**.

Table 2. Results of Vorbrüggen reactions of **29** with **67**.

Entry	Equiv. of **67**	Temp.	Time	Yield of **93**
1	1	70 °C	3 h	–
2	2	85 °C	1 day	–
3	3	80 °C	18 h	–

3.4 Synthesis of 2'-deoxy-2'-fluoroarabinonucleoside

For glycosylation of nucleobase **29** with 2-fluoroarabinofuranose **82**, nucleobase anion glycosylation using KOH and TDA-1 or Vorbrüggen conditions were applied in order to synthesize desired nucleoside **94** (**Scheme 18**).



Scheme 18. Synthesis of 2'-deoxy-2'-fluroarabinonucleoside **94**. Reagents: **(a)** 1. HBr in acetic acid, DCM 2. KOH (3 equiv.), TDA-1 (1 equiv.), MeCN; **(b)** BSA (1 equiv.), TMSOTf (2 equiv.), MeCN.

Conversion to bromose followed by nucleobase anion glycosylation was used for the synthesis of potential HCV RNA replication inhibitors[9] and 2'-deoxy-2'-fluroarabinonucleosides.[58] 1-Bromo sugar **83** was prepared by treatment of **82** with 33 % HBr in acetic acid according to literature conditions.[9] Bromose **83** was then dissolved in anhydrous MeCN and added (dropwise) to the mixture of nucleobase **29**, KOH and TDA-1 in MeCN. After 20 h stirring at RT and work-up, desired nucleoside **94** was obtained in 10 % yield. Higher temperature of the reaction (50 °C), did not significantly increase the yield (11 %). With 2 equiv. of halogenose **83** used at RT yield of nucleoside **94** was increased to 30 % and with 3 equiv. of **83** 51 % of desired product **94** was obtained. Vorbrüggen conditions were also applied. Nucleobase

29 was silylated by BSA at 80 °C for 1 h, then furanose **82** with TMSOTf was added and reaction mixture was stirred for 1 day at 80 °C, however, only trace amount of product **94** was obtained. Results are summarized in **Table 3**. Therefore nucleobase anion glycosylation proved to be the best option for sugar derivative **82**, however, there is still some space for optimalization by increasing the amount of bromose **83** or temperature around 30–40 °C.

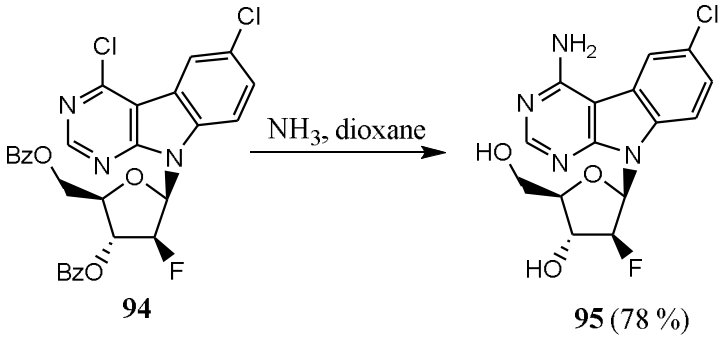
Table 3. Glycosylation of **29** with sugar derivative **82**.

Entry	Reagents	Equiv. of **82**	Temp.	Time	Yield of **94**
1	**(a)**	1	RT	20 h	10 %
2	**(a)**	1	50 °C	20 h	11 %
3	**(a)**	2	RT	20 h	30 %
4	**(a)**	3	RT	20 h	51 %
5	**(b)**	1	80 °C	1 day	<1 %

3.5 Synthesis of 4-substituted 2'-deoxy-2'-fluoroarabinonucleosides

All the active compounds from the studies[7,8] were bearing a substituent at position 4 of the pyrimidoindole system. Previous study of the regioselectivity of the Suzuki-Miyaura and Stille cross-coupling reactions resulted in fully selective formation of 4-substituted ribonucleosides. This is due to higher reactivity of the chlorine at position 4 which is caused by electron-poor nature of the pyrimidine part of the molecule, while benzene part of the molecule makes chlorine at the position 6 much less reactive. Distribution of the electrons makes this benzofused system also accessible for the nucleophilic substitution at the position 4. Despite extremely low reactivity, chlorine at position 6 was also successfully substituted and 4,6-disubstituted pyrimidoindole ribonucleosides were synthesized performing second cross-coupling reaction using X-Phos ligand in DMF.[7] However, none of the 4,6-disubstituted nucleosides showed any significant antiviral activity. The highest anti-viral activity potential in the 4-(het)aryl-6-chloro-pyrimido[4,5-*b*]indole ribonucleoside series displayed 4-amino, 4-methyl or 4-(furan-2-yl) substituted derivatives which were therefore, along with 4-(furan-3-yl) derivative, chosen for the synthesis of final nucleosides.

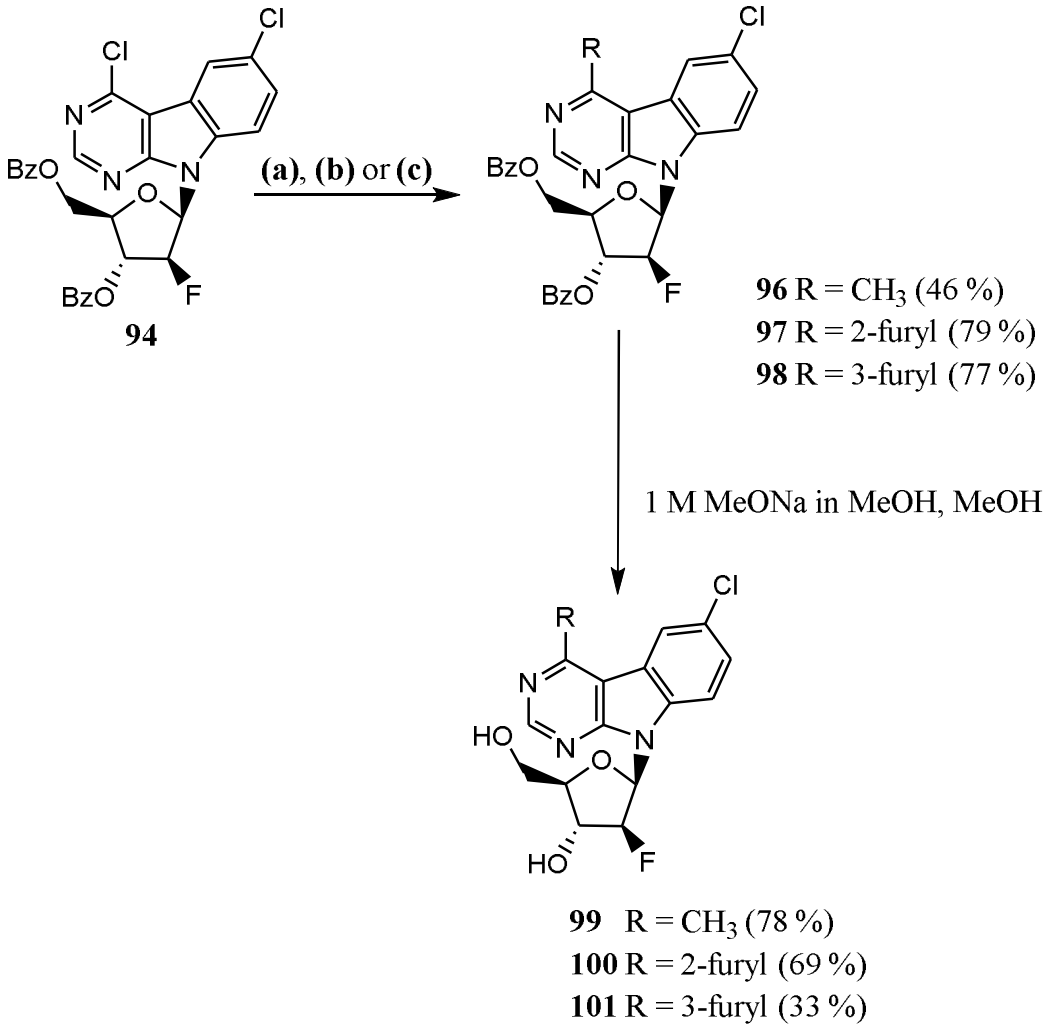
Nucleoside **94** was converted to the final deprotected 4-amino derivative **95** in 78 % yield by treatment with aqeous amonia in dioxane (**Scheme 19**).



94 **95** (78 %)

Scheme 19. Amination and deprotection of **94**.

Other substituents to the position 4 were introduced by cross-coupling reactions.[62] Protected 4-methyl nucleoside **96** was synthesized by palladium-catalyzed cross coupling reaction of **94** with trimethylaluminium in 46 % yield. Deprotection of **96** under Zemplén deacetylation conditions afforded final 4-methyl nucleoside **99** in 78 % yield. 2-Furyl derivative **97** was prepared by Stille cross-coupling with 2-(tributylstannyl)furane using $PdCl_2(PPh_3)_2$ as catalyst in 79 % yield. After deprotection, final 2-furyl nucleoside **100** was obtained in 69 % yield. 3-Furyl nucleoside **98** was obtained by Suzuki cross-coupling reaction of **94** with furan-3-yl boronic acid catalyzed by $Pd(PPh_3)_4$. Good yield (77 %) was obtained with 1 extra addition of boronic acid and catalyst, without full conversion of starting material. Deprotection of **98** aforded final 3-furyl derivative **101** (**Scheme 20**). In this case, purification using normal-phase HPFC was not successful, therefore RP-HPFC was aplied to obtain pure **101**. These two needed purifications caused low 33 % yield.



Scheme 20. Synthesis of final compounds **99**–**101**. Reagents: **(a)** $(Me)_3Al$ (2M in toluene, 2 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), THF; **(b)** 2-(tributylstannyl)furane (1.2 equiv.), $PdCl_2(PPh_3)_2$ (0.05 equiv.), DMF; **(c)** furane-3-boronic acid (1.5 equiv.), K_2CO_3 (2 equiv.) and $Pd(PPh_3)_4$ (0.05 equiv.), toluene.

4 Conclusion

Different procedures and conditions were applied for glycosylations of 4,6-dichloropyrimido[4,5-*b*]indole with ribose derivatives 2-*C*-methyl-β-D-ribofuranose, 2-deoxy-2-fluoro-α-D-arabinose and 2-deoxy-2,2-difluoro-D-ribofuranose in order to synthesize corresponding nucleosides. All attempts to synthesize 2′-β-C-methyl-ribonucleoside and 2′-deoxy-2′,2′-difluoro-β-D-ribonucleoside failed and 4,6-dichloro-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrimido-[4,5-*b*]indole was the only succesfully prepared nucleoside. Nucleobase anion glycosylation proved to be the best option for this synthesis yielding in 51 % of desired nucleoside. Series of 4-substituted derivatives of this nucleoside was prepared by nucleophilic substitution and Pd-catalyzed cross-coupling reactions in moderate to good yields (46 – 79 %). Screening of biological activities against viruses (HCV, RSV, HSV-1, HIV, Dengue, coxsackie B3 virus, influenza), cancer cell lines (HepG2, HL 60, HeLaS3, CCRF-CEM) and microbes (*Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* CCM 3953, *Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* MRSA 4591, *Staphylococcus haemolyticus* A/16568, *Escherichia coli* C/16702, *Pseudomonas aeruginosa* A/16575) is now in progress.

5 Experimental part

5.1 General remarks

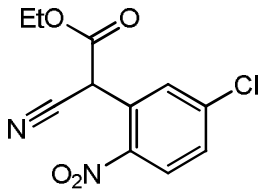
All the reagents and solvents were purchased from commercial suppliers and used as recieved. THF was dried and distilled from sodium/benzophenone. All glycosylation and cross-coupling reactions were performed under argon atmosphere.

Chromatography purifications were performed on a Biotage SP-1 apparatus with FLASH 25+M, FLASH 40+M for normal-phase or KP-C18-HS columns for reversed-phase HPFC. Merck Silica gel 60 was used for column chromatography. Monitoring of the reactions was performed using TLC Silica gel 60 F_{254} plates. Compounds were detected using shortwave (254 nm) UV lamp and solution of anisaldehyde.

NMR spectra were recorded on a 500 MHz spectrometer (^{1}H at 500 MHz, ^{13}C at 125.7 MHz and ^{19}F at 470.3 MHz), in $CDCl_3$ or DMSO-d_6. Chemical shifts were referenced to signal of TMS or DMSO and are given in ppm (δ-scale), coupling constants (*J*) in Hz. Complete assignment of all NMR signals was performed using a combination of 2D-NMR (H,H- COSY, H,C-HSQC and H,C-HMBC) experiments and configurations were established by two-dimensional ROESY spectra. Low- and high-resolution mass spectra were measured using electrospray ionization. Melting points were measured on a Stuart automatic melting point SMP40 and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were recorded on a Brucker IFS 88 spectrometer. Optical rotations were measured at 25 °C in DMSO on a Autopol IV (Rudolps Research Analytical) polarimeter, $[\alpha]_D$ values are given in 10^{-1} deg·cm^2·g^{-1}.

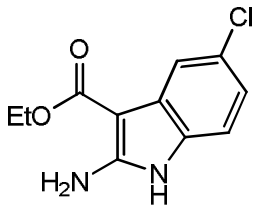
5.2 Base synthesis

Ethyl 2-(5-chloro-2-nitrophenyl)-2-cyanoacetate (89)



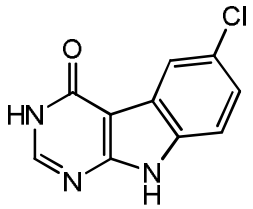
Compound **89** was prepared according to modified literature conditions.[61] Ice-cooled solution of ethyl cyanoacetate (24.6 ml; 204 mmol) in anhydrous THF under argon was treated with potassium *tert*-butoxide (23.3 g; 204 mmol). The suspension was stirred for 20 min and 2,4-dichloronitrobenzene (**88**) (20 g; 104 mmol) was added. Reaction mixture was heated to 75 °C for 1 day, then poured into water and acidified to pH~2 with 2M HCl. This mixture was extracted with ether (3x150 ml). Combined organic layers were dried over sodium sulfate and solvents were evaporated. After drying under reduced pressure compound **89** (40.6 g) was obtained as brown oil. Crude material was used directly for the next step. For analysis, the oil was purified by column chromatography (hexane/EtOAc, 0–10 % EtOAc). ^{1}H NMR is in agreement with literature.[63]

Ethyl 2-amino-5-chloro-1*H*-indole-3-carboxylate (90)



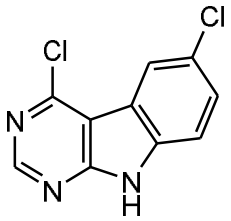
Compound **90** was prepared according to literature conditions[7] Crude **89** (40 g) was dissolved in glacial acetic acid (350 ml) and zinc dust (30 g) was added by parts in 45 min. Resulting mixture was stirred for 2 h without external heating, filtered through a pad of celite and washed with 400 ml of acetic acid. Solvent was evaporated and residue was washed with 600 ml of water. After drying under reduced pressure compound **90** (26.7 g) was obtained as brown powder. Crude material was used directly for the next step. For analysis, powder was purified by column chromatography (hexane/chloroform, 0–60 % chloroform). ^{1}H NMR is in agreement with literature.[64]

6-Chloro-3*H*-pyrimido[4,5-*b*]indol-4(9*H*)-one (91)



Compound **91** was prepared according to literature conditions.[7] Crude **90** (26.7 g) was dissolved in formamide (120 ml) and heated to 185 °C for 1 day. Cooled reaction mixture was filtered and washed with 1 l of water. After drying under reduced pressure compound **91** (14.33 g) was obtained as dark powder. Crude material was used directly for the next step. For analysis, powder was purified by column chromatogramy (chloroform/MeOH, 3 % MeOH). ^{1}H NMR is in agreement with literature.[7]

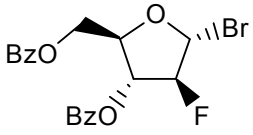
4,6-Dichloro-9*H*-pyrimido[4,5-*b*]indole (29)



Compound **29** was prepared according to literature conditions[7]. Crude **91** (14.33 g) was dissolved in $POCl_3$ (150 ml) and heated to 120 °C for 2 days. $POCl_3$ was evaporated under reduced pressure, residue was diluted with cold water and cooled with ice. Solution was slowly neutralized with aqeous ammonia to pH~7, filtered and washed with water, hydrochloric acid and again with water. After drying under reduced pressure compound **29** (7.7 g) was obtained as dark powder. Crude material was used for glycosylations without purification. For analysis, it was purified by column chromatogramy (chloroform/MeOH, 3 % MeOH). ^{1}H NMR is in agreement with literature.[7] Overall yield of 4-step synthesis of the compound **91** was 31 %.

5.3 2'-deoxy-2'-fluoro-arabinonucleosides

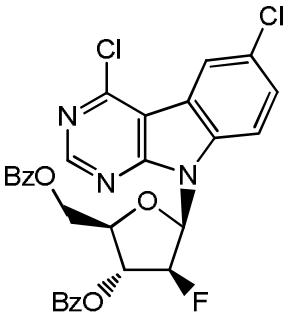
1-α-Bromo-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinose (83)



Compound **83** was prepared according to modified literature conditions.[9] HBr (33% in acetic acid; 30 ml) was added dropwise to ice-cooled solution of 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinose (**82**) (3.9 g; mmol) in anhydrous DCM (80 ml). Cooling and stirring continued for 2 h. Reaction mixture was stirred for 22 h at RT. Volatiles were removed under reduced

pressure, residue was dissolved in DCM and washed with water, saturated $NaHCO_3$ and again with water. Organic layer was dried over $MgSO_4$, filtered and solvent was evaporated. Compound **83** was obtained as light orange oil (4.24 g) and used for glycosylation without further purification. ^{1}H NMR is in agreement with literature.[65]

4,6-Dichloro-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrimido-[4,5-*b*]indole (94)



Pyrimidoindole **29** (1 g; 4.2 mmol) and finely grounded potassium hydroxide (0.71 g; 12.6 mmol) were suspended in acetonitrile (70 ml) and TDA-1 (1.34 ml; 4.2 mmol) was added. Reaction mixture was stirred for 30 min at RT, crude bromose **83** (5.12 g) in acetonitrile (55 ml) was added dropwise. Reaction mixture was stirred for another 20 h at RT. Mixture was filtered through pad of celite and volatiles were removed under reduced pressure. Crude product was purified using HPFC (hexane/EtOAc, 0–30 % EtOAc). Nucleoside **94** (1.244 mg; 51 %) was obtained as yellowish solid.

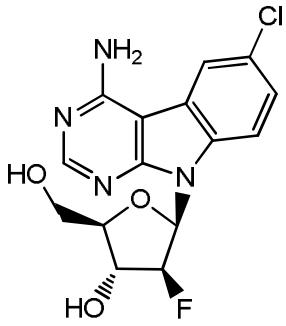
Mp 158–163 °C, IR (ATR): ν = 1716, 1273, 1114, 1096, 1071, 1029, 709 cm^{-1}.

^{1}H NMR (500.0 MHz, DMSO-d_6): 4.75 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.3, 2.9, H-4'); 4.80 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.3, H-5'b); 4.94 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 2.9, H-5'a); 5.83 (ddd, 1H, $J_{H,F}$ = 51.1, $J_{2',1'}$ = 4.1, $J_{2',3'}$ = 1.9, H-2'); 5.99 (ddd, 1H, $J_{H,F}$ = 22.5, $J_{3',4'}$ = 5.9, $J_{3',2'}$ = 1.9, H-3'); 7.13 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.17 (dd, 1H, $J_{H,F}$ = 21.4, $J_{1',2'}$ = 4.1, H-1'); 7.57, 7.60 (2 × m, 2 × 2H, H-*m*-Bz); 7.72, 7.74 (2 × m, 2 × 1H, H-*p*-Bz); 7.97 (ddd, 1H, $J_{8,7}$ = 8.9, $J_{H,F}$ = 3.2, $J_{8,5}$ = 0.5, H-8); 8.07, 8.12 (2 × m, 2 × 2H, H-*o*-Bz); 8.26 (dd, 1H, $J_{5,7}$ = 2.1, $J_{5,8}$ = 0.5, H-5); 8.93 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.16 (CH_2-5'); 76.62 (d, $J_{C,F}$ = 28.6, CH-3'); 77.70 (d, $J_{C,F}$ = 2.5, CH-4'); 83.14 (d, $J_{C,F}$ = 17.5, CH-1'); 95.38 (d, $J_{C,F}$ = 193.2, CH-2'); 111.07 (C-4a); 116.37 (d, $J_{C,F}$ = 6.0, CH-8); 119.65 (C-4b); 121.50 (CH-5); 127.26 (C-6); 128.01 (CH-7); 128.89 (C-*i*-Bz); 128.99, 129.07 (CH-*m*-Bz); 129.45 (CH-*o*-Bz); 129.47 (C-*i*-Bz); 129.87 (CH-*o*-Bz); 133.87, 134.15 (CH-*p*-Bz); 137.14 (C-8a); 152.51 (C-4); 154.80 (CH-2); 155.51 (C-9a); 165.18, 165.54 (CO-Bz). ^{19}F{^{1}H} NMR (470.3 MHz, DMSO-d_6): -189.41.

ESI MS m/z (rel.%): 602.0 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{29}H_{21}Cl_2FN_3O_5$ $[M+H]^+$: calcd 580.08357; found 580.08368.

4-Amino-6-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-pyrimido-[4,5-*b*]indole (95)



Nucleoside **94** (300 mg; 0.52 mmol) was dissolved in dioxane (2 ml) and 30% aqeous ammonia (6 ml) was added. Mixture was stirred in screw-cap pressure glass tube at 100 °C for 2 days. Volatiles were removed under reduced pressure and crude product was purified using HPFC (DCM/MeOH, 0–20 % MeOH). After recrystallization from MeOH/H_2O mixture desired product **95** (145 mg; 78 %) was obtained as white crystals.

Mp 267–272 °C; $[\alpha]_D$ 40.1 (c 0.299).

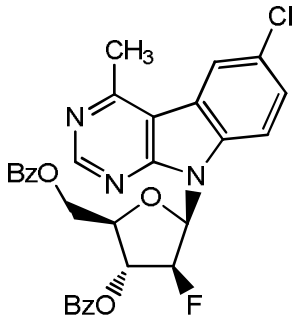
IR (ATR): ν = 3484, 3327, 3153, 2939, 1655, 1590, 1576, 1462, 1306, 1043, 797, 426 cm^{-1}.

^1H NMR (499.8 MHz, DMSO-d_6): 3.73-3.85 (m, 3H, H-4',5'); 4.46 (dddd, 1H, $J_{H,F}$ = 23.5, $J_{3',4'}$ = 5.5, $J_{3',OH}$ = 5.3, $J_{3',2'}$ = 2.4, H-3'); 5.14 (ddd, 1H, $J_{H,F}$ = 53.3, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 2.4, H-2'); 5.17 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5'); 5.92 (d, 1H, $J_{OH,3'}$ = 5.3, OH-3'); 6.84 (dd, 1H, $J_{H,F}$ = 21.1, $J_{1',2'}$ = 4.4, H-1'); 7.35 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.47 (bs, 2H, NH_2); 7.87 (dd, 1H, $J_{8,7}$ = 8.8, $J_{H,F}$ = 3.1, H-8); 8.33 (s, 1H, H-2); 8.46 (d, 1H, $J_{5,7}$ = 2.1, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 60.33 (CH_2-5'); 74.29 (d, $J_{C,F}$ = 24.1, CH-3'); 82.52 (d, $J_{C,F}$ = 17.6, CH-1'); 82.95 (d, $J_{C,F}$ = 4.6, CH-4'); 94.86 (C-4a); 98.14 (d, $J_{C,F}$ = 192.1, CH-2'); 115.43 (d, $J_{C,F}$ = 5.3, CH-8); 120.39 (CH-5); 121.77 (C-4b); 124.46 (CH-7); 126.01 (C-6); 135.33 (C-8a); 155.38 (C-9a); 155.52 (CH-2); 157.87 (C-4). $^{19}F\{^1H\}$ NMR (470.3 MHz, DMSO-d_6): -188.81.

ESI MS *m/z* (rel.%): 353.0 (85) $[M+H]^+$, 375.0 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{15}H_{14}ClFN_4O_3$ $[M+H]^+$: calcd 353.08128; found 353.08112.

4-Methyl-6-chloro-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabino-furanosyl)-pyrimido[4,5-*b*]indole (96)



Protected nucleoside **94** (300 mg; 0.52 mmol) and $Pd(PPh_3)_4$ (30 mg; 0.03 mmol) were dissolved in anhydrous THF (10 ml) and $(Me)_3Al$ (520 µl, 2M in toluene) was added. Reaction mixture was stirred at 70 °C for 18 h. Solvents were removed under reduced pressure and crude product was purified using HPFC (hexane/EtOAc, 0–40 % EtOAc). Desired product **96** (135 mg; 46 %) was obtained as yellowish solid.

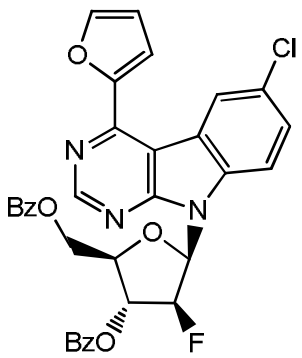
Mp 139–146 °C; IR (ATR): ν = 1721, 1477, 1454, 1264, 1164, 1115, 1101, 1072, 1043, 1031, 711 cm^{-1}.

^1H NMR (500.0 MHz, DMSO-d_6): 2.96 (s, 3H, CH_3); 4.71-4.73 (m, 1H, J_{gem} = 9.3, $J_{5'b,4'}$ = 5.2, H-5'b); 4.78 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 3.1, H-5'a); 4.94 (dd, 1H, $J_{4',3'}$ = 4.7, $J_{4',5'}$ = 4.8, 3.3, H-4'); 5.71 (ddd, 1H, $J_{H,F}$ = 21.5, $J_{3',4'}$ = 5.7, $J_{3',2'}$ = 2.5, H-3'); 6.00 (ddd, 1H, $J_{H,F}$ = 51.7, $J_{2',1'}$ = 6.5, $J_{2',3'}$ = 3.5, H-2'); 7.03 (dd, 1H, $J_{H,F}$ = 21.5, $J_{1',2'}$ = 3.5, H-1'); 7.16 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 2.2, H-7); 7.57-7.62 (m, 6H, H-*m*-Bz,-*p*-Bz); 7.91 (dd, 1H, $J_{8,7}$ = 8.3, $J_{H,F}$ = 3.5, H-8); 8.08, 8.12 (2 × m, 2 × 2H, H-*m*-Bz); 8.18 (d, 1H, $J_{5,7}$ = 2.7, H-5); 8.92 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 22.76 (CH_3); 62.95 (CH_2-5'); 76.43 (d, $J_{C,F}$ = 28.4, CH-3'); 77.25 (d, $J_{C,F}$ = 16.8, CH-1'); 82.49 (d, $J_{C,F}$ = 2.5, CH-4'); 94.67 (d, $J_{C,F}$ = 191.2, CH-2'); 111.29(C-4a); 115.61 (d, $J_{C,F}$ = 5.5, CH-8); 121.22 (C-4b); 122.05 (CH-5); 126.46 (C-6); 128.01 (CH-7); 128.74 (C-*i*-Bz); 128.82, 128.74 (CH-*m*-Bz); 128.91 (CH-*o*-Bz); 129.28 (C-*i*-Bz); 129.70 (CH-*o*-Bz); 133.71, 133.97 (CH-*p*-Bz); 136.53 (C-8a); 154.52 (C-9a); 154.52 (CH-2); 161.13 (C-4); 165.02, 165.47 (CO-Bz).

ESI MS *m/z* (rel.%): 560.0 (57) $[M+H]^+$, 582.0 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{30}H_{24}ClFN_3O_5$ $[M+H]^+$: calcd 560.13830; found 560.13841.

6-Chloro-4-(furan-2-yl)-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabino-furanosyl)-pyrimido[4,5-*b*]indole (97)



Protected nucleoside **94** (300 mg; 0.52 mmol), 2-(tributylstannyl)furane (270 mg; 0.63 mmol) and $PdCl_2(PPh_3)_2$ (18.2 mg; 0.03 mmol) were dissolved in anhydrous DMF (10 ml) and heated to 100 °C for 18 h. Solvent was coevaporated with toluene under reduced pressure. Crude product was purified using column chromatography on silica column containing 15 % of KF. Column was first washed with 3 l of hexane and then gradient of EtOAc in hexane (20 % EtOAc) was used. Desired product **97** (249 mg; 79 %) was obtained as yellow crystals.

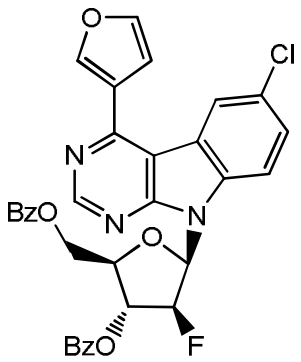
Mp 161–168 °C; IR (ATR): ν = 1723, 1439, 1266, 1252, 1090, 1071, 710 cm^{-1}.

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.89-3.91 (m, 1H, H-4'); 3.96 (dd, 1H, J_{gem} = 11.4, $J_{5'b,4'}$ = 6.1, H-5'b); 4.11 (dd, 1H, J_{gem} = 10.1, $J_{5'a,4'}$ = 3.2, H-5'a); 4.99 (ddd, 1H, $J_{H,F}$ = 42.1, $J_{2',1'}$ = 3.8, $J_{2',3'}$ = 1.9, H-2'); 5.15 (ddd, 1H, $J_{H,F}$ = 23.5, $J_{3',4'}$ = 5.6, $J_{3',2'}$ = 1.3, H-3'); 6.07 (dd, 1H, $J_{7,8}$ = 7.6, $J_{7,5}$ = 2.1, H-7); 6.19 (dd, 1H, $J_{4,3}$ = 3.1, $J_{4,5}$ = 1.6, H-4-furyl); 6.39 (dd, 1H, $J_{H,F}$ = 20.2, $J_{1',2'}$ = 5.7, H-1'); 6.73-6.78 (m, 6H, H-*m*-Bz,-*p*-Bz); 6.79 (dd, 1H, $J_{7,8}$ = 6.9, $J_{7,5}$ = 2.3, H-7); 7.11 (dd, 1H, $J_{8,7}$ = 9.2, $J_{H,F}$ = 3.3, H-8); 7.24-7.29 (m, 4H, H-*o*-Bz,8,-3-furyl); 7.52 (dd, 1H, $J_{5,4}$ = 1.5, $J_{5,3}$ = 0.8, H-5-furyl); 7.94 (d, 1H, $J_{5,7}$ = 2.5, H-5); 8.17 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.64 (CH_2-5'); 76.46 (d, $J_{C,F}$ = 23.2, CH-3'); 77.38 (d, $J_{C,F}$ = 17.9, CH-1'); 82.65 (d, $J_{C,F}$ = 4.3, CH-4'); 94.68 (d, $J_{C,F}$ = 188.1, CH-2'); 95.96 (C-4a); 106.76 (CH-4-furyl); 113.16 (CH-3-furyl); 115.60 (d, $J_{C,F}$ = 5.2, CH-8); 120.50 (C-4b); 123.39 (CH-5); 126.47 (C-6); 127.01 (CH-7); 128.74, 128.83 (CH-*m*-Bz); 128.92 (CH-*o*-Bz); 129.29 (C-*i*-Bz); 129.71 (CH-*o*-Bz); 133.72, 133.98 (CH-*p*-Bz); 137.15 (C-8a); 147.00 (CH-5-furyl); 147.95 (C-4); 152.01 (C-2-furyl); 154.41 (CH-2); 156.13 (C-9a); 165.02, 165.48 (CO-Bz).

ESI MS *m/z* (rel.%): 612.2 (70) $[M+H]^+$, 634.2 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{33}H_{24}ClFN_3O_6$ $[M+H]^+$: calcd 612.13322; found 612.13335.

6-Chloro-4-(furan-3-yl)-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabino-furanosyl)-pyrimido[4,5-*b*]indole (98)



Protected nucleoside **94** (250 mg; 0.43 mmol), furane-3-boronic acid (73 mg; 0.65 mmol), K_2CO_3 (120 mg; 0.86 mmol) and $Pd(PPh_3)_4$ (25 mg; 0,02 mmol) were dissolved in toluene (10 ml) and heated to 100 °C for 18 h. More furane-3-boronic acid (150 mg; 1.34 mmol) and $Pd(PPh_3)_4$ (45 mg; 0.04 mmol) was added and heating continued for another 2 days. Reaction mixture was diluted with water and extracted with chloroform. Organic layer was washed with saturated NH_4Cl and water, then it was dried over $MgSO_4$. Solvent was evaporated under reduced pressure and crude product was purified using HPFC (hexane/EtOAc, 0–40 % EtOAc). Desired product **98** (200 mg; 77 %) was obtained as yellow crystals.

Mp 179–187 °C; IR (ATR): ν = 1723, 1564, 1546, 1441, 1267, 1096, 1071, 803, 711 cm^{-1}.

^{1}H NMR (500.0 MHz, DMSO-d_6): 4.73-4.76 (m, 1H, H-4'); 4.80 (dd, 1H, , J_{gem} = 10.3, $J_{5'b,4'}$ = 4.7, H-5'b); 4.95 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 2.3, H-5'a); 4.51 (ddd, 1H, $J_{H,F}$ = 59.4, $J_{2',3'}$ = 5.3, $J_{2',1'}$ = 7.5, H-3'); 6.00 (ddd, 1H, $J_{H,F}$ = 59.4, $J_{2',1'}$ = 3.1, $J_{2',3'}$ = 3.7, H-2'); 7.02 (dd, 1H, $J_{H,F}$ = 24.6, $J_{1',2'}$ = 4.9, H-1'); 7.13 (dd, 1H, $J_{4,5}$ = 1.4, $J_{4,2}$ = 1.2, H-4-furyl); 7.23 (dd, 1H, $J_{7,8}$ = 7.6, $J_{7,5}$ = 3.3, H-7); 7.57-7.62 (m, 6H, H-*m*-Bz,-*p*-Bz); 7.95 (dd, 1H, $J_{7,8}$ = 7.1, $J_{7,5}$ = 2.3, H-7); 8.02 (t, 1H, $J_{8,7}$ = 9.9, $J_{H,F}$ = 4.1, H-8); 8.06-8.08 (m, 4H, H-*o*-Bz); 8.13 (dd, 2H, $J_{5,7}$ = 3.0, H-5,-5-furyl); 8.56 (dd, 1H, $J_{2,5}$ = 1.3, $J_{2,4}$ = 0.6, H-2-furyl); 9.05 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.96 (CH_2-5'); 76.45 (d, $J_{C,F}$ = 22.2, CH-3'); 77.36 (d, $J_{C,F}$ = 18.4, CH-1'); 82.62 (d, $J_{C,F}$ = 5.7, CH-4'); 94.70 (d, $J_{C,F}$ = 199.1, CH-2'); 109.90 (C-4a); 110.53 (CH-4-furyl); 115.90 (d, $J_{C,F}$ = 5.3, CH-8); 120.56 (C-4b); 121.27 (CH-5); 124.05 (CH-3-furyl); 126.20 (C-6); 126.97 (CH-7); 128.84 (CH-*m*-Bz); 128.92 (CH-*o*-Bz); 129.31 (C-*i*-Bz); 129.72 (CH-*o*-Bz); 133.72, 133.98 (CH-*p*-Bz); 136.88 (C-8a); 144.53 (CH-2-furyl); 144.78 (CH-5-furyl); 153.21 (C-4); 154.69 (CH-2); 155.41 (C-9a); 165.04, 165.49 (CO-Bz).

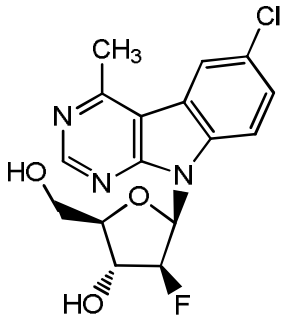
ESI MS *m/z* (rel.%): 612.0 (44) $[M+H]^+$, 634.0 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{33}H_{24}ClFN_3O_6$ $[M+H]^+$: calcd 612.13322; found 612.13348.

General procedure for deprotection of nucleosides

Protected nucleosides were dissolved in MeOH (10 ml) and 1M solution of MeONa in MeOH (1 ml) was added. Reaction mixture was stirred at RT overnight and solvent was evaporated under reduced pressure.

4-Methyl-6-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-pyrimido-[4,5-*b*]indole (99)



Deprotection of **96** (100 mg; 0.18 mmol) according to the general procedure and purification by RP-HPFC on C-18 (H_2O/MeOH, 20–60 % MeOH) afforded compound **99** (49 mg; 78 %) as white solid.

Mp 211–216 °C; $[\alpha]_D$ 33.0 (c 0.291).

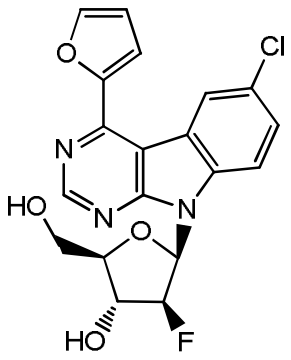
IR (ATR): ν = 3259, 2928, 2875, 1590, 1477, 1130, 1083, 814, 795, 590, 531 cm^{-1}.

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.95 (s, 3H, CH_3); 3.78 (bdd, 1H, J_{gem} = 11.9, $J_{5'b,4'}$ = 4.8, H-5'b); 3.83 (bdd, 1H, J_{gem} = 11.9, $J_{5'a,4'}$ = 3.4, H-5'a); 3.88 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.8, 3.4, H-4'); 4.50 (ddd, 1H, $J_{H,F}$ = 23.5, $J_{3',4'}$ = 5.9, $J_{3',2'}$ = 2.5, H-3'); 5.20 (bs, 1H, OH-5'); 5.21 (ddd, 1H, $J_{H,F}$ = 53.3, $J_{2',1'}$ = 4.5, $J_{2',3'}$ = 2.5, H-2'); 5.99 (bs, 1H, OH-3'); 6.96 (dd, 1H, $J_{H,F}$ = 20.5, $J_{1',2'}$ = 4.5, H-1'); 7.55 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.1, H-7); 8.04 (dd, 1H, $J_{8,7}$ = 8.9, $J_{H,F}$ = 3.0, H-8); 8.18 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.90 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 22.91 (CH_3); 60.23 (CH_2-5'); 74.22 (d, $J_{C,F}$ = 24.0, CH-3'); 82.51 (d, $J_{C,F}$ = 17.6, CH-1'); 83.12 (d, $J_{C,F}$ = 4.8, CH-4'); 98.08 (d, $J_{C,F}$ = 192.1, CH-2'); 111.35 (C-4a); 116.31 (d, $J_{C,F}$ = 5.2, CH-8); 121.18 (C-4b); 121.97 (CH-5); 126.48 (C-6); 127.10 (CH-7); 137.05 (C-8a); 154.40 (C-9a); 154.59 (CH-2); 161.03 (C-4). ^{19}F{^{1}H} NMR (470.3 MHz, DMSO-d_6): -188.81.

ESI MS m/z (rel.%): 352.1 (18) $[M+H]^+$, 374.1 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{16}H_{16}ClFN_3O_3$ $[M+H]^+$: calcd 352.08592; found 352.08587.

6-Chloro-4-(furan-2-yl)-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-pyrimido-[4,5-*b*]indole (100)



Deprotection of **97** (212 mg; 0.35 mmol) according to the general procedure and purification using HPFC (DCM/MeOH, 0–10 %) afforded compound **100** (97 mg; 69 %) as white solid.

Mp 75–81 °C; $[\alpha]_D$ 41.3 (c 0.271).

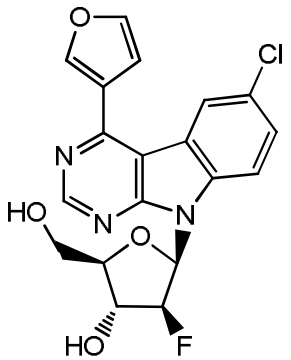
IR (ATR): ν = 3181, 2918, 1470, 1446, 1073, 1054, 1040, 1013, 793, 743 cm^{-1}.

^1H NMR (499.8 MHz, DMSO-d_6): 3.77-3.92 (m, 3H, H-4',5'); 4.51 (dddd, 1H, $J_{H,F}$ = 23.7, $J_{3',4'}$ = 5.8, $J_{3',OH}$ = 5.4, $J_{3',2'}$ = 2.4, H-3'); 5.20 (t, 1H, $J_{OH,5'}$ = 5.4, OH-5'); 5.24 (ddd, 1H, $J_{H,F}$ = 53.3, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 2.4, H-2'); 5.99 (d, 1H, $J_{OH,3'}$ = 5.4, OH-3'); 6.91 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.8, H-4-furyl); 7.03 (dd, 1H, $J_{H,F}$ = 21.0, $J_{1',2'}$ = 4.4, H-1'); 7.60 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.62 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl); 8.08 (dd, 1H, $J_{8,7}$ = 8.9, $J_{H,F}$ = 3.2, H-8); 8.36 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.9, H-5-furyl); 8.80 (d, 1H, $J_{5,7}$ = 2.2, H-5); 9.00 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 60.21 (CH_2-5'); 74.27 (d, $J_{C,F}$ = 24.2, CH-3'); 82.68 (d, $J_{C,F}$ = 17.6, CH-1'); 83.23 (d, $J_{C,F}$ = 4.7, CH-4'); 98.13 (d, $J_{C,F}$ = 192.1, CH-2'); 106.88 (C-4a); 113.32 (CH-4-furyl); 115.63 (CH-3-furyl); 116.30 (d, $J_{C,F}$ = 5.4, CH-8); 120.46 (C-4b); 123.37 (CH-5); 126.54 (C-6); 127.70 (CH-7); 137.71 (C-8a); 147.07 (CH-5-furyl); 147.98 (C-4); 152.29 (C-2-furyl); 154.50 (CH-2); 156.19 (C-9a). $^{19}F\{^1H\}$ NMR (470.3 MHz, DMSO-d_6): -188.43.

ESI MS *m/z* (rel.%): 404.0 (45) $[M+H]^+$, 425.9 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{19}H_{16}ClFN_3O_4$ $[M+H]^+$: calcd 404.08079; found 404.08078.

6-Chloro-4-(furan-3-yl)-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-pyrimido-[4,5-*b*]indole (100)



Compund **98** (164 mg; 0.27 mmol) was deprotected according to the general procedure and purified first by HPFC (DCM/MeOH, 0–10 %) and then by RP-HPFC on C-18 (H_2O/MeOH, 20–60 % MeOH) affording compound **101** (37 mg; 33 %) as white solid.

Mp 208–214 °C; $[\alpha]_D$ 58.9 (c 0.268).

IR (ATR): ν = 3266, 2926, 1593, 1477, 1296, 1057, 807, 602 cm^{-1}.

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.76-3.87 (m, 2H, H-5'); 3.90 (m, 1H, H-4'); 4.51 (m, 1H, H-3'); 5.20 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5'); 5.24 (ddd, 1H, $J_{H,F}$ = 53.4, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 2.5, H-2'); 6.00 (d, 1H, $J_{OH,3'}$ = 5.2, OH-3'); 7.02 (dd, 1H, $J_{H,F}$ = 20.6, $J_{1',2'}$ = 4.4, H-1'); 7.14 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.57 (dd, 1H, $J_{7,8}$ = 9.0, $J_{7,5}$ = 2.1, H-7); 8.02 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl); 8.07 (dd, 1H, $J_{8,7}$ = 9.0, $J_{H,F}$ = 3.1, H-8); 8.08 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.56 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 9.04 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 60.21 (CH_2-5'); 74.23 (d, $J_{C,F}$ = 24.1, CH-3'); 82.64 (d, $J_{C,F}$ = 17.5, CH-1'); 83.22 (d, $J_{C,F}$ = 4.6, CH-4'); 98.10 (d, $J_{C,F}$ = 192.1, CH-2'); 109.98 (C-4a); 110.73 (CH-4-furyl); 116.57 (d, $J_{C,F}$ = 5.1, CH-8); 120.49 (C-4b); 121.21 (CH-5); 124.28 (C-3-furyl); 126.23 (C-6); 127.61 (CH-7); 137.41 (C-8a); 144.60 (CH-2-furyl); 144.89 (CH-5-furyl); 153.17 (C-4); 154.75 (CH-2); 155.44 (C-9a). $^{19}F\{^{1}H\}$ NMR (470.3 MHz, DMSO-d_6): -188.62.

ESI MS m/z (rel.%): 404.1 (32) $[M+H]^+$, 426.1 (100) $[M+Na]^+$.

HR MS (ESI) for $C_{19}H_{16}ClFN_3O_4$ $[M+H]^+$: calcd 404.08089; found 404.08079.

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